



Selenium neurotoxicity in humans: Bridging laboratory and epidemiologic studies[☆]



Marco Vinceti^{a,b,*}, Jessica Mandrioli^c, Paola Borella^{a,b}, Bernhard Michalke^d, Aristidis Tsatsakis^e, Yoram Finkelstein^f

^a Environmental, Genetic and Nutritional Epidemiology Research Center (CREAGEN), Department of Diagnostic, Clinical and Public Health Medicine, University of Modena and Reggio Emilia, Modena, Italy

^b Trace Element Institute for Unesco Satellite Center, Department of Diagnostic, Clinical and Public Health Medicine, University of Modena and Reggio Emilia, Modena, Italy

^c Department of Neuroscience, University of Modena and Reggio Emilia and Local Health Unit of Modena, Modena, Italy

^d Research Unit Analytical BioGeoChemistry, Helmholtz Zentrum München – German Research Center for Environmental Health GmbH, Munich, Germany

^e Department of Forensic Sciences and Toxicology, University of Crete, Heraklion, Greece

^f Neurology and Toxicology Service and Unit, Shaare Zedek Medical Center, Jerusalem, Israel

HIGHLIGHTS

- Acute overexposure to the metalloid selenium (Se) is neurotoxic in the human.
- Neurotoxicity of chronic Se overexposure needs to be better characterized.
- Chronic Se overexposure might induce lethargy and amyotrophic lateral sclerosis.
- The different Se chemical species strongly vary in their neurotoxic effects.
- Peripheral biomarkers of Se exposure may not reflect Se central nervous system levels.

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ABSTRACT

Selenium is a metalloid of considerable interest in the human from both a toxicological and a nutritional perspective, with a very narrow safe range of intake. Acute selenium intoxication is followed by adverse effects on the nervous system with special clinical relevance, while the neurotoxicity of long-term overexposure is less characterized and recognized. We aimed to address this issue from a public health perspective, focusing on both laboratory studies and the few epidemiologic human studies available, with emphasis on their methodological strengths and limitations. The frequently overlooked differences in toxicity and biological activity of selenium compounds are also outlined. In addition to lethargy, dizziness, motor weakness and paresthesias, an excess risk of amyotrophic lateral sclerosis is the effect on the nervous system which has been more consistently associated with chronic low-level selenium overexposure, particularly to its inorganic compounds. Additional research efforts are needed to better elucidate the neurotoxic effects exerted by selenium overexposure.

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1. Introduction

The intense debate on the role of the metalloid selenium (Se) in human health encompasses cancer etiology (Dennert et al., 2011; Vinceti et al., 2013b), diabetes mellitus (Stranges et al., 2010; Koyama et al., 2013), amyotrophic lateral sclerosis (ALS) (Vinceti et al., 2012), and 'Keshan' cardiomyopathy (Lei et al., 2011; Li et al., 2012), alongside other infectious and non-communicable diseases. Se effects on human health may be both beneficial (Rayman, 2000) and detrimental (Vinceti et al., 2001), and the safe range of dietary Se intake is still uncertain and controversial, as shown by the most recent epidemiologic evidence and by the various standards issued

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* Corresponding author at: CREAGEN – Università di Modena e Reggio Emilia, Via Campi 287, 41125 Modena, Italy. Tel.: +39 059 2055481; fax: +39 059 2055483.

E-mail address: marco.vinceti@unimore.it (M. Vinceti).

by different agencies (Vinceti et al., 2009; Fairweather-Tait et al., 2011; Hurst et al., 2013; Vinceti et al., 2013a). Recent field and laboratory studies have added to this ambiguity, thus further hampering the risk assessment of this metalloid and the definition of permitted limits of environmental exposure, and preventing consensus on public health policy (Vinceti et al., 2013a, 2013b).

Among the intriguing aspects are the role of Se in the etiology of neurological disease (Vinceti et al., 2001, 2009), also taking into account the complex peculiarities of Se physiopathology and metabolism in the brain (Buckman et al., 1993; Pullen et al., 1995; Whanger, 2001; Bou-Resli et al., 2002; Schweizer et al., 2004; Scharpf et al., 2007; Benner et al., 2013). Unfortunately, the relation between Se exposure and neurological diseases has been addressed in few human studies, in some cases affected by relevant methodological limitations, and therefore it necessitates further validation and extension. A number of studies also suggested or evidenced the key importance of additional factors of interest when assessing Se biological activity and toxicity, such as the chemical form of Se, and the concurrent exposure to other toxic chemicals (i.e. mixed exposures) (Gammelgaard et al., 2011; Michalke and Berthele, 2011; Zwolak and Zaporowska, 2012; Solovyev et al., 2013; Vinceti et al., 2013c; Weekley and Harris, 2013).

In this review, we have briefly analyzed Se neurotoxicity on the basis of the scarce epidemiologic evidence available, also considering the biological plausibility of findings from laboratory and veterinary medicine studies and the recent interest in Se neurotoxicity in risk assessments of metals and metalloids (Fresquez et al., 2013). For the selection of the literature eligible for this review, we examined in detail PubMed-indexed papers using as MeSH search terms “Nervous System Diseases” associated with “selenium/toxicity”. Moreover, we systematically scanned PubMed to retrieve the human and laboratory studies on Se investigating its neurotoxicity. Some caveats need however to be outlined. First of all, a relation between Se and neurological disease calls into question not only its toxicity but also its nutritional role. In fact, both an increase and a decrease in the amount of bioavailable Se might theoretically enhance the risk of neurological disease and its progression. The hypothesis that an increased Se intake may reduce the risk of diseases such as Alzheimer’s disease or amyotrophic lateral sclerosis (ALS), or counteract their clinical progression has been evaluated in laboratory studies (Scharpf et al., 2007; Bellinger et al., 2012; Raman et al., 2012), some of which indicated beneficial effects of organic and inorganic Se compounds in experimental models of neurodegenerative diseases (Schweizer et al., 2004; van Eersel et al., 2010; Wirth et al., 2010; Zhang et al., 2010; Caito et al., 2011; Dasuri et al., 2013). However, no such effects have been confirmed by human investigations. Moreover, our review did not analyze the possible inverse relation between Se status and psychiatric disorders, a currently controversial issue (Berr et al., 2012; Gao et al., 2012; Hurst et al., 2013; Miller et al., 2013).

2. Laboratory studies on Se neurotoxicity

The neurotoxic effects of Se have long been investigated in laboratory studies (Kasuya, 1976; Ammar and Couri, 1981; Rasekh et al., 1997, 1998) and several recent studies on this issue have been published (Xiao et al., 2006; Ayaz et al., 2008; Morgan et al., 2010; Souza et al., 2010; Maraldi et al., 2011; Estevez et al., 2012). One of the pioneering studies on Se neurotoxicity showed the ability of both inorganic and organic Se compounds to induce behavioral and neurological manifestations in mice, with selenite being much more toxic than selenomethionine (Ammar and Couri, 1981). In this investigation, Se species induced a decrease in locomotion followed by ataxia and hind limbs paralysis and dysfunction, generalized muscular flaccidity and catalepsy-like

state; respiratory and heart rates also markedly decreased, and were followed by death due to respiratory and cardiac arrest.

The neurotoxic effects inducible by Se compounds include among others an increase of central nervous system (CNS) dopamine levels (Rasekh et al., 1997) and metabolites (Tsunoda et al., 2000), alteration of cholinergic signaling and degeneration of cholinergic neurons (Estevez et al., 2012), inhibition of glutamate uptake (Nogueira et al., 2003; Ardais et al., 2010; Souza et al., 2010) and prostaglandin D synthase (Islam et al., 1991; Matsumura et al., 1991; Akarsu et al., 1998; Ardais et al., 2010), decrease of total antioxidant status, gangliosides and sulphhydryl groups (Islam et al., 2004; Medeiros et al., 2012), of activity of adenosine deaminase (Bitencourt et al., 2013), succinic dehydrogenase and acetylcholine esterase (Nehru and Iyer, 1994), and finally increase of thiobarbituric acid reactive substances and lipid peroxidation (El-Demerdash, 2001; Islam et al., 2004; Glaser et al., 2010; Medeiros et al., 2012). Additional Se-induced CNS alterations are hypothermic and nociceptive responses as well as CNS arousal (Mallory Boylan et al., 1990; Rasekh et al., 1998), and reduction of locomotor activity (Rasekh et al., 1997, 1998; Morgan et al., 2010). Finally, inorganic Se has also been shown to induce apoptosis in cultured mouse cortical neurons even at very low concentrations (Xiao et al., 2006). Some of these effects are differentially exerted in various CNS regions, even with opposite mechanisms (Zia and Islam, 2000; Islam et al., 2004; Glaser et al., 2010; Medeiros et al., 2012). Se-induced neuromuscular blockade, tetanic spasm, alteration of nerve-fiber action potentials and nerve membrane depolarization (Liu et al., 1989; Lin-Shiau et al., 1990; Ayaz et al., 2008), and inhibition of human squalene monooxygenase, which may in turn lead to peripheral demyelinating neuropathy (Gupta and Porter, 2002), are all additional findings from experimental studies with potential clinical implications.

The neurotoxicity of Se compounds is also manifested by its ability to induce degeneration of motor neurons. In the study by Maraldi et al. (2011), human neuroblastoma SKNBE cells were shown to be more prone than other human cell lines to the neurotoxicity of inorganic and organic Se compounds: the lowest effects on viability were observed at levels as low as 8 µg/l. Moreover, Se induced a broad range of intracellular effects including increased intracellular levels of reactive oxygen species, inducible nitric oxide synthase and 3-nitrotyrosine, and superoxide dismutase-1 translocation from the cytosol to the mitochondria, the latter phenomenon characterizing the neurodegenerative process in the ALS form associated with superoxide dismutase-1 mutation. In another study, inorganic tetravalent Se, selenite, induced degeneration of cholinergic neurons and depletion of glutathione, impairing locomotor activity in *Caenorhabditis elegans* model (Morgan et al., 2010; Estevez et al., 2012). The cholinergic motor neurons in the ventral cords exhibited several neurodegenerative signs following Se exposure: axonal beading, cellular swelling and nuclear cytoplasmic boundary loss and fragmentation. Se disrupted the orderly array of presynaptic densities in this region, as previously observed at the neuromuscular junction in a superoxide dismutase-1 mouse model (Fischer et al., 2004). Moreover, veterinary research on inorganic and organic Se poisoning in swine showed acute neuromuscular signs with progressive posterior paralysis and in some cases forelimb involvement, progressing to lateral recumbence and death (Harrison et al., 1983; Wilson et al., 1983; Anonymous, 2010; Nathues et al., 2010; Raber et al., 2010). These findings were obtained both in observational studies following accidental acute and chronic Se intoxication, and experimentally by administering Se-accumulator plants and various Se forms (Hartley et al., 1984; Panter et al., 1996). Pathological findings were selective degeneration of the ventral horns in the spinal cord, bilateral poliomyelomalacia in the cervical and lumbar/sacral spinal cord

intumescences, loss of neurons with reactive vascular proliferation and glial phagocytic cell response, alongside the degeneration of brain stem motor nuclei (Harrison et al., 1983; Wilson et al., 1983). Such Se-induced neurotoxic effects have not been reported in other animal species, with the exception of a study in cattle that evidenced a similar effect (Maag et al., 1960), thus corroborating the observation in farm animals and wildlife of seleniferous areas of the so-called 'alkali disease' and 'blind staggers' (Rosenfeld and Beath, 1964). The experimental studies in swine showed that the inorganic Se species selenite and selenate were more neurotoxic than organic Se compounds at equivalent levels of Se exposure (Panter et al., 1996), regardless of the higher Se levels in tissues following exposure to organic Se compounds. Remarkably, Se is the only element and more generally the only chemical, as far as we know, which may be selectively toxic to the motor neurons, suggesting biological plausibility for its potential role in the etiology of ALS, though such effect might strongly differ in different living organisms.

Overall, experimental studies have shown different toxic effects of various inorganic and organic Se compounds (Borella et al., 1996; Hoefig et al., 2011; Nogueira and Rocha, 2011; Bitencourt et al., 2013; Boehler et al., 2013; Hazane-Puch et al., 2013), as suggested by epidemiologic studies (Ashton et al., 2009; Vinceti et al., 2013a, 2013b, 2013c). The neurotoxicity of inorganic Se may exceed the neurotoxicity of organic Se (Ammar and Couri, 1981) by more than 40-fold. The differential toxicity and metabolism of various organic and inorganic Se forms makes it strongly improper to generalize the term 'Se neurotoxicity', whereas each neurotoxic effect should be referred to as a specific poisoning by a specific Se compound. An extensive effort should be made in the future to address the issue of Se neurotoxicity in humans by assessing the specific effects of certain Se species.

An issue of interest is the ability of Se to counteract the toxic effects, mainly the neurotoxicity, of other elements. A large body of evidence suggested an inhibitory effect exerted by Se against heavy metal neurotoxicity, e.g. mercury (Wang et al., 2013), lead (Nehru and Iyer, 1994; Liu et al., 2013), cadmium, and aluminum neurotoxicity. However, not all the results were consistent, and sometimes Se served as an agonist in cases of mixed poisoning (Kasuya, 1976; Glaser et al., 2010). The ability of Se to form complexes with other toxic elements in various organs including the CNS might also induce a longer persistence of the elements, which is of some concern as it possibly leads to long-term release of Se and of heavy metals (Bjorkman et al., 1995) in the brain.

In conclusion, these above-mentioned experimental studies point to several and divergent mechanisms of Se neurotoxicity, which however may not necessarily be relevant to humans, considering species-related susceptibility and the differences between acute, subacute or chronic exposures in laboratory studies and long-term low-level exposures in human lifetime.

3. Relevance of Se speciation for CNS and exposure assessment methods

It is now clear that the impact of Se on the organism is strongly dependent on its chemical species, as shown by a large number of studies concerning the nutritional and toxicological properties of the metalloid in cell cultures and living organisms (Borella et al., 1996; Michalke et al., 2009; Vinceti et al., 2009; Hazane-Puch et al., 2013; Weekley and Harris, 2013). The impact of the various Se species on neuronal health, however, is still largely unknown and very controversial, since in contrast with toxicologic studies most molecular biological investigations using knock-out mice or cell culture experiments revealed neuroprotective action of Se compounds such as selenoprotein P-bound Se (SePP),

glutathioneperoxidase-bound Se (GPx), and thioredoxinreductase-bound Se (TrxR) (Wirth et al., 2010; Schweizer et al., 2011; Dasuri et al., 2013). The studies suggested an important role of selenoproteins in the maintenance of optimal brain functions via redox regulation, showing among other effects neuronal and axonal degeneration after SePP depletion, the restoration of dopaminergic neurotransmission by selenite, and mitigation of tau pathology by selenate (Khan, 2010; van Eersel et al., 2010; Zhang et al., 2010; Caito et al., 2011; Raman et al., 2012). Only a few Se species were however investigated in these investigations, typically in an on/off approach and rarely in a concentration-dependent manner.

This puzzled picture drawn from the literature about Se-caused neuroprotection versus neurodegeneration triggered probably (mainly) from elevated inorganic Se species, further highlights the needs for Se speciation studies including species identification and quantification when assessing Se neurotoxicity and more generally Se effects on CNS (Michalke et al., 2009; Solovyev et al., 2013; Vinceti et al., 2013c). Moreover, such studies should advisably be conducted in samples relevant to brain-Se metabolism, in order to avoid inappropriate conclusions about the impact of Se on neuronal health or disease without reference to its compound-specific concentration in the CNS.

Terms related to chemical speciation have been published by Templeton et al. (2000). In these guidelines, elemental speciation is linked to a quality-controlled clear species identification and quantification of all species of an element present in a representative sample. A literature survey on Se speciation in neuronal relevant tissue or body fluids revealed that today such papers are scarce. Michalke and Berthele (2011) published a first snapshot of Se speciation in human cerebrospinal fluid (CSF) after a preceding study of this group had demonstrated the independence of CSF-Se from serum-Se, hence pointing to a strict Se-species regulation in the brain and/or regulated transport across neural barriers (Dasuri et al., 2013). Six Se species were quantified of which SePP, TrxR, human serum albumin-bound Se and selenate could be identified, while two more Se-peaks remained unidentified. A follow-up study investigated paired serum/CSF samples to enlighten the possible transport of Se-species across neural barriers (Solovyev et al., 2013). Se-species were quantified in both sample types (as $\mu\text{g/l}$ for serum # CSF) as selenoprotein P (5.19 # 0.47), Se-methionine (0.23 # <LoD), GPx (4.2 # 0.036), TrxR (1.64 # 0.035), selenite (12.25 # 0.046) and human serum albumin-bound Se (18.03 # 0.068). In comparison to other papers on Se species in serum or plasma (Zhang et al., 2010) in that reference serum SePP was found at somewhat lower level, while GPx was at similar concentration. However, the results from paired samples demonstrated strong differences not only between total Se concentrations and serum, but more importantly between individual Se-species concentrations from CSF and serum. Strikingly, strong correlations between the two paired sample types were found only for GPx ($r^2 = 0.6636$) and TrxR ($r^2 = 0.8031$), resulting in calculated Q-values (conc-CSF vs. conc-serum) of 8.3×10^{-3} for GPx or 21.3×10^{-3} for TrxR. Both values were considerably increased compared to the albumin value of 5.25×10^{-3} being in the normal range for healthy neural barriers of this age group. This increase of Q-values was explained by their facilitated diffusion or transport across neural barriers or their independent expression in the brain. Interestingly, no correlation was found between serum and CSF content of the inorganic Se species selenite and selenate, of the organic form SePP, and of overall Se (Solovyev et al., 2013; Vinceti et al., 2013c), indicating the inability of peripheral indicators such as blood (or nails, urine and hair) to assess Se exposure in specific compartments such as the CNS. This may indicate the inadequacy of commonly used biomarkers to assess Se exposure, and the potential role of individual factors related to Se metabolism, possibly under genetic control, in determining Se CNS content.

4. Human studies: acute Se intoxication

Reports about acute Se intoxication include suicide attempts, consumption of Se-containing dietary supplements, intake of food sources with very high Se content like Brazil nuts, occupational exposures and rarer etiologies (Vinceti et al., 2001; Nuttall, 2006; Morris and Crane, 2013). In one of the first studies on acute Se exposure, 5 workers out of 25 were noted as affected by progressive dizziness and severe lassitude after adding Se to the ink which they used (Buchan, 1947). In another report, a 22 year old female biology student died after the suicidal ingestion of a sodium selenate solution (Lech, 2002): post-mortem examination showed cerebral edema. Localized or generalized tremor (Ransone et al., 1961; Sioris et al., 1980) and convulsions (Carter, 1966; Civil and McDonald, 1978) were also shown to be prominent symptoms of acute Se intoxication.

A high occurrence of fatigue, irritability, and peripheral neuropathy followed the ingestion of over-the-counter tablets (in the US) that contained 27,300 µg of Se, 182-fold higher than labeled (Helzlsouer et al., 1985). Fatigue and paresthesias were also reported following the consumption of a misformulated Se supplement, hypothesized to contain high amount of organic Se (Clark et al., 1996).

A recent detailed study on acute Se intoxication enrolled 97 subjects accidentally exposed to misformulated dietary supplements containing over 40,000 µg of Se as selenate, 200-fold the intended dose (Morris and Crane, 2013), a preparation which caused the severest Se toxicity outbreak ever occurred in the US, involving 201 cases (MacFarquhar et al., 2010). The biomarkers of acute exposure to this inorganic hexavalent Se species were monitored, along with the long-term health effects of this exposure during a 2.5 year-follow-up in 73 subjects (Morris and Crane, 2013). Toenail Se levels were first determined 4 months after consuming misformulated Se supplements, showing much higher values than the restored-baseline concentrations, and also strongly correlated with total Se intake during the exposure period. Analyses performed serially in subjects' toenail specimens showed an increase of Se content over time, with a peak median lapsed time of 237 days after the last exposure and a median time to restored-baseline concentrations of 411 days. The overall Se consumption by subjects ranged between 669,570 and 965,520 µg in 30 days, a dose exceeding more than 400 times the recommended dietary allowance in the US (55–70 µg/day). Questionnaire data indicated a high occurrence of dermatological lesions that usually follow Se overexposure, and about half as many of the subjects manifested long-term neuropsychological signs and symptoms: fatigue, confusion, memory loss, anxiety, fingertip tingling, depression, anger, irritability, insomnia, dizziness and imbalance, eye and vision problems and headache. Tremors also occurred but in a lower number of subjects (20% approximately). The occurrence of ataxia was not investigated in this study, but this sign had been observed in 13% of 201 patients exposed to such Se-containing misformulated supplement in a previous report (MacFarquhar et al., 2010). The natural history of this acute Se toxicity is peculiar: in most cases (57.1%), an improvement of symptoms was reported after 2.5 years, whereas 33.3% of the study group reported no improvement and 9.5% reported worsening, thus contradicting previous reports on shorter recovery periods (Morris and Crane, 2013). This different natural history might be ascribed specifically to the ability of selenate to induce persistent neurotoxic sequelae after acute intoxication. Some limitations of this study must also be highlighted and were acknowledged by the authors. The investigation was undertaken in response to a Se toxicity outbreak, had limited statistical power, and lacked a control group. There was no independent validation of health symptoms that had been self-reported: however, the exceedingly high prevalence of symptoms supports their authenticity.

Moreover, in the 27 study subjects for whom both peak toenail Se concentrations and self-reported symptoms were available, some evidence of a direct correlation, though statistically imprecise, emerged ($r=0.256$; $p=0.2$ – courtesy of John Steven Morris, University of Missouri, unpublished data). A longer-term follow-up of this population would be of considerable interest to assess the risk of chronic diseases, including the neurological ones.

In summary, neurotoxicity in humans is highly prevalent, long lasting and probably irreversible after acute Se poisoning, particularly for some Se species. However, most studies have not systematically analyzed the neurological effects, and in some studies no such effects were noted. Therefore, the relations between acute Se poisoning and neurological diseases, as well as the possible effects of chronic poisoning by low-level exposures, require further epidemiologic investigation, as is needed for the differential neurotoxic effects of various Se compounds. An additional issue is the possibility to identify common underlying toxicological mechanisms of Se acute adverse effects on the nervous, dermal and endocrine systems.

5. Human studies: chronic exposures to environmental Se

Neurotoxicity following chronic Se overexposure, considerably more common than acute exposure, has been investigated by very few epidemiologic studies, significantly differing from each other by their design and population characteristics, and the overall picture emerging from these investigations is incomplete and not entirely consistent. Unfortunately, most studies carried out in populations overexposed to environmental Se have not investigated long-term neurological effects.

One of the first and pioneering studies on the health effects of chronic Se overexposure was carried out in China and specifically in Hubei Province, characterized by a very high soil Se content particularly in its Enshi district (Yang et al., 1983; Zheng et al., 1992). The local residents consumed locally-grown food, and largely used locally-produced coal. Unusual signs and symptoms of Se poisoning were observed in this population (Yang et al., 1983; Zheng et al., 1992; Yang and Zhou, 1994; Li et al., 2012). Neurological signs were found in 18 out of 22 rural residents affected by severe selenosis: the clinical picture included acroparesthesia and dysesthesia (“pins and needles”), hyperreflexia, convulsions, motor weakness and hemiplegia, abnormalities which were ascribed to ‘polyneuritis’ caused by Se intoxication. However, this diagnosis does not appear to be adequate to explain all symptoms detected, such as hyperreflexia and convulsions, which are at least in part due to CNS involvement. Moreover, there was unfortunately no direct information on the chemical forms of Se implicated in the excess environmental exposure investigated in this study, though the main source of exposure was diet, which is expected to contain almost entirely organic Se. However, emissions from coal combustion and consumption of contaminated drinking water may have contributed to the Se exposure in that area, providing inorganic Se in such case (Finkelman et al., 1999; Gujian et al., 2007; Vinceti et al., 2013a).

During a subsequent survey carried out in the Enshi district by the geologist Fiona Fordyce of the British Geological Survey, clinical data on Se poisoning among rural residents made available by local public health officials were collected and reported, in addition to a large body of environmental data about the features of Se contamination (Fordyce, 1996, 2007). A variety of neurological signs and symptoms defined as ‘no strength in limbs’, ‘tingling limbs’ and ‘paralysis’ were found in a variable range of 1–5% among 180 subjects from villages in which Se toxicity occurred (Fordyce, 1996). However, relevant methodological details were not reported, such as the sampling methodology, the extent of Se exposure in affected

individuals, the Se species responsible of such intoxication, and the exact rates of neurological signs and symptoms among the individuals investigated. Moreover, potential confounding from age, gender, behavioral and environmental factors was not assessed, and no control group was provided. Despite these limitations, the study provided evidence supporting a relation between chronic Se overexposure and neurotoxic effects.

In a study carried out in the United States by Valentine et al., health status was assessed among 50 residents in three communities with unusually high Se content (mean = 494, 194 and 327 $\mu\text{g/l}$) in their tap water (Valentine et al., 1987; Valentine, 1997). The control group included 99 residents in two communities with drinking water containing 2–3 $\mu\text{g Se/l}$. The exact nature of Se compounds was not reported, but it seems reasonable to assume that Se was inorganic, probably selenate (Hu et al., 2009; Kuisi and Abdel-Fattah, 2010). Blood and hair Se levels were moderately elevated, and urinary Se was considerably elevated in the highly exposed residents. The prevalence of neurological diseases (“diseases of the nerves, paralysis or numbness”), examined only in the age group 18–55 and for participants who were users of tap water, was limited but it tended to be higher in the exposed group (3/50, 6%) compared with the reference group (1/99, 0.5%), and this was also true when subjects with higher Se status (defined as urine Se > 70 $\mu\text{g/day}$ or blood Se > 120 $\mu\text{g/l}$) were investigated and compared with residents with the lower exposure (5.4% vs. 1.7% using urine Se as an indicator, 4.9% vs. 1.8% using blood Se) (Valentine et al., 1987). A broad spectrum of neurological symptoms (depression, dizziness, musculoskeletal pain and headache) was more frequent in residents in the exposed communities compared with unexposed populations, and this was confirmed in the subgroup with higher Se exposure according to biomarkers. However, the evaluation of these comparisons was difficult due to the small sample size of the groups investigated.

The risk of neurological symptoms was estimated in 142 inhabitants of areas with endemic Se overexposure from South Dakota and Wyoming (Longnecker et al., 1991), having a median Se daily intake of around 200 μg . No effect of Se exposure on the risk of paresthesias was found (it actually decreased). By contrast, an increased risk of lethargy emerged since the odds ratio (OR) of having this sign more frequently than the median for an increase of one standard deviation of whole blood, toenails, or dietary Se was equal to 1.41 (95% confidence interval (CI) 1.01–1.96), 1.41 (1.02–1.95), and 1.43 (0.98–2.09), respectively, and a slight excess risk of muscle twitches (OR: 1.17 (0.84–1.64), 1.10 (0.80–1.51), and 1.28 (0.87–1.88)) and dizziness (OR: 1.20 (0.88–1.64), 1.29 (0.94–1.76), and 1.17 (0.82–1.66)) was also noted. The authors also stated that the ‘statistical significance’ of the association between Se exposure and lethargy decreased after excluding one influential observation from the analysis, or adjusting for rancher-nonrancher status, but they did not report in detail the relevant ORs with their 95% CIs.

A unique situation of chronic exposure (1974–1988) to drinking water with a high content (around 8 $\mu\text{g/l}$) of selenate of geologic origin was studied in the Rivalta neighborhood in Reggio Emilia, Italy. Drinking water in the rest of municipal neighborhoods contained Se levels far below 1 $\mu\text{g/l}$, as usual in underground water in Italy and elsewhere (Vinceti et al., 1998, 2000, 2010). After fixing the local problem of such high Se level too close to the upper standard of 10 $\mu\text{g/l}$ (Vinceti et al., 2013a), Se water levels in Rivalta decreased to less than 1 $\mu\text{g/l}$. The analysis of educational attainment level and occupation in the cohorts consuming the high- and low-Se tap water showed a comparable profile (Vinceti et al., 1995), an observation that along with the very similar chemical composition of their tap waters apart from Se made it possible to define the study setting as a natural experiment, usually of strong interest in environmental epidemiology (Rothman et al., 2008). This setting

thus allowed to investigate a potentially toxic exposure, inorganic Se (nearly absent in foodstuffs (Combs, 2001)), also minimizing the risk of bias from confounding. The occurrence of neurological diseases was investigated in the cohorts of Rivalta residents, using mortality and, where possible, incidence, as end-points of interest during 9–12 years of follow-up. Two neurodegenerative disorders, Parkinson’s disease and ALS, showed an excess mortality (Vinceti et al., 1995, 2000) based on three deaths for each disease: the inclusion of these two diseases was first done non-specifically, considering only the causes of death for which an increased mortality emerged. Further validation of ALS risk was done through two incidence studies (Vinceti et al., 1996, 2010), whose design and implementation was prompted not only by the original results of the Rivalta mortality studies but also by the original description of a cluster of the disease associated with excess Se exposure in the US. Such investigation was the report of a cluster of ALS, including four cases of the disease, in a seleniferous region of South Dakota (Kilness and Hochberg, 1977). Additional evidence supporting the Se-ALS association was provided by laboratory and veterinary medicine studies which showed a selective motor neuron toxicity of some Se compounds in swine (Vinceti et al., 2012, 2013c). The indication of an excess risk for ALS (and Parkinson’s disease) from the Rivalta studies suggested therefore a major concern, to be further evaluated in populations exposed to high levels of inorganic Se (Vinceti et al., 2013a). Despite the low statistical precision of the estimates due to the small numbers of observed cases, this study also contributed to the suggestion of a precautionary reassessment of the current safe upper limit of Se level in drinking water (Vinceti et al., 2013a). Interestingly, early-onset alopecia has been recently associated to a higher risk of subsequent onset of ALS (Fondell et al., 2013), an observation is of interest since alopecia is a typical effect of Se overexposure even at low doses (Vinceti et al., 2001; Nuttall, 2006; Lippman et al., 2009), although alopecia clearly has many possible causes including a single nucleotide polymorphism variant in the region of gene TAR DNA-binding protein 43, also suggested to be implicated in ALS pathogenesis (Fondell et al., 2013).

A study on 448 residents aged 15–87 years in 12 communities in the Brazilian Amazon tested the hypothesis that Se exposure, as assessed through several biomarkers of exposure, could affect motor functions (Lemire et al., 2011). High-level exposure to Se and mercury (Hg) in these populations derived from the consumption of a Se-rich diet of Brazil nuts, fish species, meat and eggs (Lemire et al., 2012), and the median plasma Se level resulted to be 135 $\mu\text{g/l}$. The study results showed a direct association between Se plasma levels and motor performance, while simultaneously controlling Hg and lead (Pb) blood levels. These results appear to disprove the detrimental effect of Se exposure on motor functions, but may also be due to confounding, such as unmeasured heavy metals (other than Hg and Pb) and other chemicals. Moreover, the study did not address exposure to specific Se compounds, although they were most likely organic because of their dietary origin. Finally, the results may be irrelevant to ALS, a rapidly progressive and extremely severe disease which cannot be directly compared to mild to moderate declines in motor functions.

The peculiar sensitivity of children to adverse neurotoxic effects of Se was addressed in 102 Canadian Inuit children aged 5–6 years. Umbilical cord levels of several contaminants were measured at their birth. The high consumption of fish and marine mammals by this population was associated to an unusually high intake of polychlorinated biphenyls, methyl-Hg, Se and other potentially neurotoxic substances (Saint-Amour et al., 2006), with Se umbilical blood level being 429 $\mu\text{g/l}$ on the average. Measurements of pattern-reversal visual evoked potentials (VEP) N75, P100 and N150 were conducted to assess developmental neurotoxicity. VEP exhibited longer latencies, suggesting optic nerve demyelination

as a consequence of elevated Se blood levels on the visual system, even after adjusting for potential confounders such as methylmercury in multivariate analysis. Thus, high Se intake during childhood might have a negative impact on the visual system and not be protective against methylmercury toxicity, suggesting the occurrence of subclinical effects at high Se blood levels. Clearly, these effects at young age may be different from what can be observed at older age, and the possibility of confounding or effect modification by other contaminants should also be considered (Saint-Amour et al., 2006; Yang et al., 2013).

Another study performed neurobehavioral assessment in a cohort of 927 3-day old Chinese neonates using a Neonatal Behavioral Neurological Assessment score for functional abilities based on 6 indexes: behavior, passive tone, active tone, primary reflexes and general assessment (Yang et al., 2013). Both a direct correlation of the score with Se cord levels <100 µg/l and an inverse association for higher Se levels were found, suggesting the occurrence of an inverse U-shaped relation between this behavioral and neurological assessment and Se exposure. These results indicate an extremely narrow margins of safety of Se exposure in neonates, possibly suggesting higher susceptibility to Se neurotoxicity in the early developmental period (Yang et al., 2013).

Other environmental Se overexposures have been described, but neurological issues do not unfortunately appear to have been investigated in the seleniferous areas spread throughout the world such as in Venezuela, Mexico, and India (Brätter et al., 1991; Brätter and Negretti de Brätter, 1996; Vinceti et al., 2001; Hira et al., 2004; Hurtado-Jimenez and Gardea-Torresdey, 2007; Dhillon and Dhillon, 2009).

The investigation of chronic occupational exposures is another potential approach to assess health effects of chronic Se exposure, but such exposures in workers appear to be uncommon, and moreover neurotoxicity following long-term Se exposure in occupational settings has been rarely investigated. Such analyses of health effects of Se exposure in occupational settings may also be of considerable interest since it may involve 'rare' exposure to inorganic volatile Se compounds, specifically released in such environments. We are aware of two investigations which evaluated the consequence of chronic exposure. Holness et al. (1989) assessed health status in 40 Se-exposed copper refinery workers and 150 controls: a few neurological symptoms were more prevalent in the exposed individuals, including dizziness, sleep disturbances and particularly paresthesias. The latter symptom was reported by 29%, 35% and 45% of the 31, 23 and 29 Se-exposed workers examined in three consecutive visits, respectively, compared with a rate of 3% in a control group including 150 individuals. Stiffness, fatigue and muscle-joint pain were also found as strongly increased in the Se-exposed workers. In another occupational study, weakness and fatigue were found to be considerably more prevalent in 19 workers who were exposed to Se during the manufacture and maintenance work of drums used in photocopy machines, compared with a control group of 15 unexposed workers (Srivastava et al., 1997).

An alternative approach to assess the risk of neurological diseases associated with Se exposure has been the implementation of case-control and cross-sectional studies based on biomarkers. Se blood and tissue levels in neurological patients and controls were measured in several studies, even though some of them were of limited size and did not control for potential confounding factors. These studies addressed Alzheimer's disease (Ceballos-Picot et al., 1996; Loef et al., 2011), Parkinson's disease (Qureshi et al., 2006; Younes-Mhenni et al., 2013) and ALS (Mitchell et al., 1991; Ince et al., 1994; Markesbery et al., 1995; Vinceti et al., 1997; Bergomi et al., 2002; Vinceti et al., 2013c). Major methodological limitations, however, affected these investigations. First, exposure assessment of Se was based on indicators such as toenails or blood Se levels, which may be unreliable in assessing Se burden in the CNS and

possibly other target organs (Solovyev et al., 2013; Vinceti et al., 2013c). Moreover, these studies investigated the overall Se content in biological fluids, disregarding the specific exposures to the different Se compounds and the complex patterns which may arise from Se speciation studies. For example, in a recent investigation in newly diagnosed ALS patients (thus minimizing a disease-induced effect on Se biomarkers) and matched hospital controls, in which the CSF content of the various Se compounds was measured, levels of organic Se species were lower but concentrations of selenite and human serum albumin-bound Se levels were higher in ALS patients (Vinceti et al., 2013c). In general, most of the case-control and cross-sectional studies assessing overall Se exposure in patients with neurological diseases suffered from severe risk of biases such as selection bias, inadequate exposure assessment, confounding, and reverse causality. To yield reliable information of etiologic importance, studies using organ-specific indicators of exposure to single Se compounds should be carried out, though the complexity of such studies limits their feasibility.

6. Moving forward: research priorities and precautionary risk assessment for Se neurotoxicity

Current epidemiologic evidence in the human unambiguously shows the neurotoxicity of acute Se exposure and also appears to support such effects following low-level chronic Se overexposure, although the latter relation is still inadequately characterized. The biological plausibility of Se neurotoxicity is also clearly supported by laboratory and veterinary medicine evidence. The results of the few human studies conducted on this issue as well as their limitations, as described above, call for further investigation of Se neurotoxicity, focusing on the effects of long-term low-level Se overexposure as well as the specific activity of the various Se species. Additional evidence regarding these issues is needed also to better assess the safe range of Se exposure, which is still controversial.

Conflict of interest

None declared.

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