A case report of motor neuron disease in a patient showing significant level of DDTs, HCHs and organophosphate metabolites in hair as well as levels of hexane and toluene in blood

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ABSTRACT

Motor neuron disease is a devastating neurodegenerative condition, with the majority of sporadic, non-familial cases being of unknown etiology. Several epidemiological studies have suggested that occupational exposure to chemicals may be associated with disease pathogenesis. We report the case of a patient developing progressive motor neuron disease, who was chronically exposed to pesticides and organic solvents. The patient presented with leg spasticity and developed gradually clinical signs suggestive of developing progressive motor neuron disease, who was chronically exposed to pesticides and organic solvents. The concentration of non-specific dialkylphosphates metabolites (DAPs) of OPs in hair (dimethylphosphate (DMP) 1289.4 pg/mg and diethylphosphate (DEP) 709.4 pg/mg) and of DDTs (opDDE 484.0 pg/mg, ppDDE 526.6 pg/mg, opDDT 448.4 pg/mg and ppDDT 573.7 pg/mg) were considerably significant. Toluene and n-hexane were also detected in blood on admission at hospital and quantified (1.23 and 0.87 μg/l, respectively), while 3 months after hospitalization blood testing was found negative for toluene and n-hexane and hair analysis was provided decreased levels of HCHs, DDTs and DAPs.

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Introduction

Motor neuron disease (MND) is a group of neurodegenerative disorders selectively affecting motor neurons. Its most common and well characterized form is amyotrophic lateral sclerosis (ALS), a devastating disease caused by relentless degeneration of motor neuron populations in the motor cortex, brainstem and spinal cord grey matter and resulting in progressive disability with death occurring 1–5 years from disease onset. Its etiology remains largely unknown. About 10% of ALS cases are considered to be familial and a number of related genes have been recognized (Valdmanis and Rouleau, 2008). For the vast majority of sporadic cases, the factors contributing to disease pathogenesis are still under investigation. Epidemiological studies have proposed that exposure to certain chemicals, including pesticides and organic solvents, might increase the risk of developing ALS to exposed populations (Sutedja et al., 2009). However, in these studies exposure assessment is mostly based on self-reporting and does not provide information about the particular chemical agents possibly implicated.

In the present article, we report the case of a patient developing progressive motor neuron disease, with significant levels of DDTs and organophosphates in hair as well as levels of organic solvents (n-hexane and toluene) in blood. While MND associated with repeated high level exposure to organochlorine pesticides (Fonseca et al., 1993) and with acute poisoning by both pyrethroids and organochlorines (Pall et al., 2009)
has been reported in the past, this is to our knowledge the first case where exposure to both organochlorine and organophosphate pesticides as well as chronic occupational exposure to organic solvents has been related to development of ALS.

Case report

The patient, a 57-year-old male furniture maker, had a 3-month history of lower back pain that evolved to sciatica and painful muscle cramps that involved the muscles of the pelvic region and the thighs bilaterally. One month prior to his first hospital admission (June 2010), the patient noticed a gradually deteriorating gait difficulty. He reported unexplained weight loss of about 17 kg (~20% of his initial body weight) within the 3 months preceding the onset of his symptoms. Past medical history was unremarkable except for familial essential tremor and paroxysmal atrial fibrillation. He has been a heavy smoker since early adulthood (approximately 80 pack-years).

On neurologic examination, marked gait spasticity due to severely increased muscle tone, more evident in the lower extremities, very brisk reflexes (++++) in all four extremities and extensor plantar responses bilaterally were found. Although muscle strength was normal, there was moderate atrophy involving the muscles of the shoulder region (mainly the trapezius and suprascapular muscles) bilaterally, more pronounced on the left side. The remainder of the detailed examination of cranial nerves, sensory and cerebellar function was normal.

Complete blood count and chemistry values, thyroid function testing, vitamin B12 and folate levels were normal. Assays for antinuclear antibodies and other immunologic tests, including onconeural antibodies, were negative. Lumbar puncture yielded acellular cerebrospinal fluid with normal glucose and moderately elevated protein levels (103.4 mg/dl). Evaluations for infectious agents in serum and CSF, including bacterial, fungal, and viral sources, were normal. Magnetic resonance imaging (MRI) of the cervical and lumbar spine revealed no abnormal findings, whereas in the MRI of the brain, high density signal along the course of the corticospinal tracts bilaterally in T2-weighted and FLAIR images was evident (Fig. 1). Transcranial magnetic stimulation of the cortex revealed delayed central conduction for the lower extremities and the left upper extremity. Nerve conduction studies showed normal motor and sensory nerve conduction velocities, F-wave latencies and compound muscle action potential amplitudes without conduction block in all limbs. Needle electromyography disclosed giant motor units and decreased recruitment of motor units in the left 1st dorsal intersosseous and polyphasic motor unit potentials in trapezius and supraspinatus bilaterally and left sternocleidomastoid, indicative of chronic denervation changes. In addition, signs of active denervation (fibrillation potentials and positive sharp waves) were detected in the right trapezius. The patient’s condition worsened over the next 6 months. He was unable to walk independently due to increasing spasticity and mildly diminished muscle strength in both legs. Atrophy of the shoulder region muscles was more prominent, accompanied by moderate muscle weakness of the rotator cuff muscles.

On detailed history taking, our patient reported using, on a regular basis, various paints for his work, some diluted in organic solvents, for the last 30 years. He also reported spraying his workshop extensively with insecticides almost every month. While performing these tasks, he never used protective equipment. However, he never experienced any signs/symptoms of acute solvent or pesticide toxicity after the aforementioned actions, even in winter, when windows of his poorly ventilated workshop were kept closed.

Therefore, a detailed list of all preparations recently used by the patient for furniture painting and pest control was sought. The diverse preparations used for varnishing (paints and diluting solutions) contained toluene, xylene, polyisocyanates and polyurethane in varying concentrations. The most frequently used diluant, a general purpose nitro-thinner, contained hydrotreated naphtha, methyl acetate, toluene, methanol and acetone. Acrylic paints contained methacrylic esters. The glue used for his work contained polyvinylacetate. An ethanol preparation, containing also turpentine oil, was used by the patient daily for cleaning his hands. He also reported occasionally using acetone as well as formaldehyde for various diluting and cleaning purposes. For pest control, the two preparations used on an almost monthly basis in the recent past contained Phoxim 50% w/v (which rapidly metabolized to DEP) (Robert and Huston, 1999) and Flufenoxuron 9.6% w/v respectively. Additives included 4-Methylpentan-2-on, Butan-1-ol, N-methyl-2-pyrrolidone, cyclohexaneone and ethoxylated nonylphenol phosphate. However, he had used numerous preparations in the past, most of which he was not able to recall.

Materials and methods

Sample collection. Blood (2 ml) and hair (~300 mg) samples were collected during (5 cm) and 3 months after hospitalization (3 cm). Hair samples were removed from the back of the head close to the scalp and stored appropriately in paper envelope, in ambient temperature until treatment and analysis.

Blood analysis for volatile organic solvents. One (1) milliliter of whole blood or blank blood spiked with hexane and toluene (at spiked levels

Fig. 1. Axial fluid attenuation inversion recovery (FLAIR) magnetic resonance imaging of the brain. An increased signal is shown along the course of the corticospinal tracts bilaterally (arrows) within the posterior limb of the internal capsule (A) and within the cerebral peduncles (B).
from 0 to 5 μg/l) was mixed with 1 ml of LCMS grade water and transferred into 10 ml headspace vials. The vials were shaken well, rested for 10 min and placed in an oil bath at 80 °C for 30 min. An aliquot of 250 μl headspace air was injected into the GC-MS system (splitless mode) and analyzed under the following temperature conditions: The column temperature was initially held at 110 °C for 5 min, raised to 180 °C at a 10 °C/min rate and held for 2 min. Helium flow rate was 0.95 ml/min. The injector, interface and ion source temperatures were 210 °C, 250 °C and 220 °C respectively, while the mass spectrometer was operated at the selected ion-monitoring mode. The Wiley7 library was used for the qualitative determination of n-hexane (m/z = 41, 43 and 57) and toluene (m/z = 65, 91, 92) with similarity above than 94%.

Persistent pollutants and non-specific organophosphate metabolites extraction from hair. The extraction of persistent pollutants (DDTs, hexachlorocyclohexanes-HCHs and polychlorinated biphenyls-PCBs) and DAPs was performed based on previous published methods. Briefly, methodologies are presented below.

DDTs, HCHs and PCBs extraction. Head hair sample (100 mg) was washed with water and hexane and cut in small pieces (approximately 2–3 mm). Then, 2 ml hexane was added in each sample and incubating for 2 hours in 40 °C. This step was repeated two times. The quantities of hexane (3 × 2 ml) were collected and moved to clean up step by SPE cartridges. Cartridges were activated by the addition of 2 ml of hexane: dichloromethane (4:1, v/v). For elution, the solvent used was 2 ml of hexane: dichloromethane (1:1, v/v). The final eluate was dried under a gentle nitrogen stream and reconstituted in 70 μl heptane (Tsatsakis et al., 2008a, 2008b).

DAPs extraction. Hair (100 mg) was washed twice in 5 ml of water and methanol. Washed hair samples were dried and pulverized in a ball mill homogenizer. Two (2) milliliters of methanol and dibutyl phosphate (DBP, as internal standard) were added and hair was incubated at room temperature in an ultrasonic bath for 4 hours. Liquid–solid extraction was followed for 30 min. The mixture was centrifuged at 4000 rpm for 5 min, filtered and transferred to a test-tube containing 15 mg of K₂CO₃ and methanol was evaporated to dryness. Fifteen (15) milligrams of K₂CO₃ was added again to the residue, reconstituted in 1 ml of acetonitrile and 0.1 ml solution of pentafluorobenzylbromide (PFBBr) in acetonitrile (1:3, v/v) and incubated at 80 °C in a water bath for 30 min. The acetonitrile was evaporated to dryness and the residue was dissolved in 50 μl of toluene (Tsatsakis et al., 2010).

GC-MS analysis. The analysis of organochlorine (α-HCH, HCB, lindane, opDDE, ppDDE, ppDDD, ppDPT, ppDDT), polychlorinated biphenyl (PCB 28, 52, 101 and 118) and DAPs, DMP, DEP, diethylthiophosphate (DETP) and diethylidithiophosphate (DDETP) was performed on a electron ionisation mass spectrometric GC-QP2010 Shimadzu system equipped with a Equity TM-5 (30 m × 0.20 mm × 0.20 μm) capillary column supplied by Supelco (Supelco, 595 North Harrison Road, Bellefonte, PA 16823-0048, USA). Pure helium with a flow rate was 0.95 ml/min as a carrier gas. Solution was injected into the system in the splitless mode and analyzed under the following conditions:

For DDTs and HCHs. The column temperature was initially held at 60 °C for 1 min, raised to 180 °C at 15 °C/min, held for 1 min, raised to 250 °C at 4 °C/min, held for 1 min and was finally raised to 300 °C, at 30 °C/min, where it remained stable for 2 min.

For PCBs. The column temperature was initially held at 60 °C for 1 min, raised to 300 °C at 10 °C/min and held for 1 min. The injector temperature was 270 °C for DDTs and HCHs and 230 °C for PCBs analysis. The interface ion source temperatures and was set at 310 °C and 220 °C respectively. The mass spectrometer was operated at the selected ion-monitoring mode.

For DAPS. The column temperature was initially held at 60 °C for 1 min, raised to 180 °C at 20 °C/min, held for 1 min, raised to 250 °C at 4 °C/min, held for 1 min and was finally raised to 300 °C, at 25 °C/min, where it remained stable for 2 min. The injector, interface and ion source temperatures were set at 270 °C, 300 °C and 230 °C, respectively.

Methodology for the MRI. The patient underwent brain MRI on a 1.5 T whole-body scanner (Vision/ Sonata, Siemens/Erlangen), equipped with high performance gradients (Gradient strength: 40 mT/m, slew rate: 200 mT/m/ms) and a standard quadrature head coil. The basic protocol comprised of the following sequences: a) 3D T1-w (MPRAGE, TR 1570/TE 1.73 ms, 1 mm³/1 NEX/160 axial slices), b) T2-w TSE (TR/TE = 5000/98 ms) with 4-mm axial sections, and c) TSE-FLAIR (TR/TE/TI = 9000/120/2600 ms) with 4-mm axial and sagittal sections. Axial sections were acquired parallel to the plane connecting the anterior and posterior commissures (AC-PC lines). For all conventional scans uniform geometry parameters were used (256 field of view and an acquisition matrix of 256 × 256).

Results

The levels of HCHs, DDTs, PCBs and the analyzed DAPs are shown in Table 1. For α-HCH, HCB and lindane, the detected concentration levels in the hair sample collecting during hospitalization were 63.0, 123.4 and <20 pg/mg of hair (total HCHs 186.4 pg/mg). For opDDE, ppDDE, ppDDD, ppDPT + opDPT, ppDPT were 484.0, 526.6, 448.4, 275.9 and 573.7 pg/mg respectively (total DDTs 2308.6 pg/mg). For DMP, DEP, DETP and DDETP were 1284.9, 709.4, 186.4 and 33.6 pg/mg respectively (total DAPs 2218.8 pg/mg). No quantified levels of PCB (28, 52, 101 and 118) were detected in head hair sample with a limit of quantification 0.6 pg/mg.

Table 1

Concentrations of organochlorine pollutants (HCHs, DDTs and PCBs), of non-specific metabolites of organophosphate pesticides (DAPs) in head hair samples as well as of n-hexane and toluene in blood samples, collected during (1st sample) and 3 months (2nd sample) after hospitalization.

<table>
<thead>
<tr>
<th>Concentration (pg/mg)</th>
<th>HCHs</th>
<th>a-HCH</th>
<th>HCB</th>
<th>Lindane</th>
<th>Total HCHs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st sample</td>
<td>63.0</td>
<td>123.4</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>186.4</td>
</tr>
<tr>
<td>2nd sample</td>
<td>12.1</td>
<td>37.1</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>49.2</td>
</tr>
<tr>
<td>DDTs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st sample</td>
<td>484.0</td>
<td>526.6</td>
<td>448.4</td>
<td>275.9</td>
<td>573.7</td>
</tr>
<tr>
<td>2nd sample</td>
<td>175.0</td>
<td>245.3</td>
<td>111.2</td>
<td>75.1</td>
<td>187.9</td>
</tr>
<tr>
<td>PCBs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st sample</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>2nd sample</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>DAPs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st sample</td>
<td>1289.4</td>
<td>709.4</td>
<td>186.4</td>
<td>33.6</td>
<td>2218.8</td>
</tr>
<tr>
<td>2nd sample</td>
<td>130.1</td>
<td>415.3</td>
<td>211.2</td>
<td>41.2</td>
<td>797.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration (μg/l)</th>
<th>Solvents</th>
<th>n-hexane</th>
<th>Toluene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st sample</td>
<td>0.87</td>
<td>1.23</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>2nd sample</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
</tbody>
</table>

For PCB lines). For all conventional methods of PCB lines. For all conventional methods of PCB lines. For all conventional methods of PCB lines.

*LOQ = 0.20 μg/mg for lindane.

*LOQ = 0.06 μg/mg for PCBs.

*LOQ = 0.5 μg/l for toluene and 0.35 μg/l for hexane.
In the second hair sample, collected 3 months after hospitalization, reduced levels of DAPs (total DAPs 797.8 pg/mg), HCHs (total HCHs 49.2 pg/mg) and DDT (total DDTs 794.5 pg/mg) were qualified.

The head space analysis of blood sample provided 1.23 and 0.87 μg/l of toluene and n-hexane respectively (Table 1). Blood sample received 3 months after hospitalization was found negative for toluene and n-hexane (with limit of quantification (LOQ) for toluene and n-hexane 0.5 and 0.35 μg/l respectively).

Discussion

Epidemiological studies have implicated various classes of chemicals, such as heavy metals, organic solvents and pesticides, to be related with a higher risk for sporadic ALS (Sutedja et al., 2009).

Regarding exposure to solvents, while some case-control studies have suggested a positive association with the risk for sporadic MND/ALS development (Chancellor et al., 1993; Gunnarson et al., 1992; Gunnarson and Lindberg, 1989; Mitchell et al., 1995; Morahan and Pamphlett, 2006; Sienko et al., 1990), other studies did not confirm these suggestions (Gait et al., 2003; McGuire et al., 1997).

Pesticides are strong candidates because they are ubiquitously used and can be neurotoxic to humans through various mechanisms. Acute OPs poisoning can cause a broad spectrum of signs and symptoms, including dizziness, headache, nausea, tremor, convulsions, muscle weakness, coma and death. OPs, accounting for the majority of studied acute intoxication cases, are thought to exert their neurotoxic effects mainly through acetylcholinesterase and other esterase inhibition (Kamel and Hoppin, 2004; Vilanova et al., 1999).

The acute toxic effects of overexposure to organochlorines have also been described previously (Moses and Peter, 2010; Taylor et al., 1978). Regarding the mechanism of organochlorine neurotoxicity, effects of DDTs are mediated mainly through modulation of sodium channel opening (Hong et al., 1986; Ishikawa et al., 1989), while other classes of organochlorine compounds are thought to be neurotoxic due to blocking of the GABA-gated chloride channel (Casida, 1993) or due to dysregulation of intracellular signaling systems (Kodavanti, 2005). In addition to their acute neurotoxic effects, previous studies suggested that chronic low level exposure to pesticides can lead to a variety of chronic neurologic conditions, ranging from slight deterioration in cognitive and psychomotor function to progressive neurodegenerative disorders. While many studies have reported an association between pesticide exposures and Parkinson’s disease (Kamel and Hoppin, 2004) with the possible pathogenic mechanisms involved being extensively studied (Franco et al., 2010), the association of pesticide toxicity to ALS is still under investigation.

A big confound in the studies reporting pesticide exposures and diseases is the multitude of types of chemicals that are used as pesticides. Even within classes, different chemicals can have different effects. Whereas some population studies suggested an association of agricultural occupations with higher ALS/MND prevalence (Govoni et al., 2005; Gunnarson et al., 1991; Holloway and Emery, 1982; Rosati et al., 1977), other studies did not confirm such relationship (Buckley et al., 1983; Leone et al., 1983). Inconsistent results also have been noted for the association between exposure to pesticides and risk for sporadic ALS, with several case-control studies reporting a significantly increased risk among people exposed to pesticides (Bonvicini et al., 2010; McGuire et al., 1997; Morahan and Pamphlett, 2006; Savettieri et al., 1991), whereas other studies resulted in non-significant trends (Chancellor et al., 1993; Granieri et al., 1988; Gunnarson et al., 1992). A recent large prospective study, thus eliminating recall bias, found an increased risk of ALS associated with exposure to formaldehyde, but only a non-significant trend with respect to pesticides and no association with exposure to other organic chemicals and solvents (Weisskopf et al., 2008). However, limitations of the latter study were that it was based on self-reported exposure and there was a lack of information about the frequency and intensity of exposures.

Reasons for these inconsistencies are not yet clear. One possible explanation is that all these studies are prone to recall bias, since they are retrospectively designed and rely either on self-reporting or on assumption of exposure based on occupational history to assess exposure to pesticides, solvents and other chemicals. In addition, either the duration or the intensity of exposure sometimes was lacking or not systematically reported. Finally, as ALS is a rare disease, the number of cases available for study is a limiting factor, making statistical power to identify associations low.

Although, as discussed above, epidemiological data suggest that pesticide neurotoxicity could be a contributing factor to MND development, only two case reports have been published to this date, in which MND/ALS developed after exposure to pesticides, thus suggesting a relationship. In the first one, Pall et al. (1987) reported a patient developing rapidly progressive ALS 2 weeks after a single episode of high level exposure to a chlordane and permethrine based insecticide. Nevertheless, determination of pesticide concentrations in body samples was not performed. In the second report, Fonseca et al. (1993) described two patients, a farmer and his employee, who had repeatedly been preparing and spraying organochlorine pesticide solutions during some agricultural seasons, and both developed MND. Notably, the patients reported preparing pesticide solutions with bare hands before each use. Furthermore, at least one of those patients had experienced signs and symptoms of acute organochlorine intoxication in the past, for which he had been hospitalized twice. Very high levels of organochlorine pesticides were measured in peripheral blood in both patients, interestingly, not the same compounds: aldrin was detected in the first, while lindane and heptachlor were detected in the other. An ALS-like disease following chronic heavy exposure to pyrethroids was recently published by Doi et al. (2006). However, the patient, who fulfilled clinical and electrophysiological criteria for ALS on presentation, significantly improved after cessation of exposure to pesticides, thus suffering from a non-progressive condition.

Our patient showed upper and lower motor neuron clinical signs, the former being more pronounced during the initial stage of his evolving illness. In addition, neurophysiologic studies indicated upper and lower motor neuron involvement, whereas the involvement of pyramidal tracts was also seen in the brain MRI, thus fulfilling the El Escorial criteria for ALS diagnosis.

The patient was chronically exposed to chemicals by frequent contact through his skin and by inhalation during most of his working life: on a daily basis to solvents and on an almost monthly basis to pesticides. Increased levels of DDT and its metabolites as well as of DAPs were found in hair sample, whereas the presence of n-hexane and toluene was detected in peripheral blood. The levels of DDTs were much higher than those reported in a previous study in the general and occupational exposure population (Tsatsakis et al., 2008b). Tsatsakis and co-authors reported median (max, 1st–3rd quartile) concentrations levels of a-HCH, HCB, lindane, opDDE, ppDDE, opDDD, ppDDD + ppDPT and ppDDT in head hair samples of the population of this region at 7.2 pg/mg (50.3, 2.7–10.9), 2.2 pg/mg (15.9, 1.2–5.9), 70.2 pg/mg (174.7, 48.2–95.0), 2.7 pg/mg (571, 0.9–7.2), 5.7 pg/mg (58, 1.4–33.3), 3.1 pg/mg (6.8, 2.5–3.5), 2.6 pg/mg (2135.0, 1.1–14.1), 23.2 pg/mg (158.7, 0.5–90.2), respectively.

Covaci (Covaci et al., 2008) also noted that ppDDT degraded to ppDDD and ppDDE inside the body as well as ppDDE shows high accumulation potential and long biological half-life. As has been reported, a ratio of ppDDE/ppDDD lower than 5 indicates recent exposure to parent DDT (Jaga and Dharmani, 2003). In our study the corresponding ratio was 0.9 suggesting recent exposure to parent DDT. The significant detected levels of DDTs probably due to past exposure to those compounds or to the high burden to DDT of the working place (Neuber et al., 1999) as it is known that DDT has large accumulation potential and long biological half-life. Moreover the loss of the 20% of the body weight might
have a crucial role to the increased levels of DDTs in hair samples. The fact that, in the second hair sample, obtained 3 months after hospitalization, the levels of DDTs had already dropped significantly, is compatible with this hypothesis: While the first hair sample of 5 cm length is roughly representing hair growth during the period of symptom onset, which was preceded by the patient’s weight loss, the second hair sample (3 cm length), obtained 3 months after the initial presentation of the patient, represents hair growth of a time period during which his body weight had already been stable for more than 3 months.

Our analytical data suggest the use of OPs producing DMP (e.g. dichlorvos, methyl parathion, dimethoate) and DEP (e.g. chlorpyrifos, ethion, diazinon, phoxim) (Tsatsakis et al., 2010). The detected concentration values of DMP was high (1289.4 pg/mg) as well as of DEP (709.4 pg/mg) and are comparable to median levels previously reported for occupational exposure population (812.9 pg/mg. 447.8–995.3 1st–3rd quartile) (Tsatsakis et al., 2010). On the other hand, the levels of DETP (186.4 pg/mg) and DEDTP (33.6 pg/mg) were in the range given for the general population (non-occupational) exposure (median value and 1st–3rd 54.0 pg/mg (21.3–84.9) and 40.0 (33.6–78.6) pg/mg for DETP and DEDTP respectively) (Tsatsakis et al., 2010).

Although the concentrations of n-hexane and toluene in blood confirm exposure of the patient to organic solvents, they reflect neither the level nor the duration of exposure to these substances. The concentration found in blood are lower than those (from 183 μg/l to 207 μg/l) reported in studies investigating exposure to certain levels of n-hexane in inspired air (360 mg/m3) (Veulemans et al., 1982) or to occupational exposure to toluene (car mechanics) (from 0.338 to 4.539 μg/l) (Schimming et al., 1999). In contrast to the aforementioned case reports, our patient was not subject to acute high level exposure, but to repeated, low level occupational exposure to DDTs and OPs for many years, as judged by the detailed history and the laboratory analyses. The question is whether or how this chronic occupational exposure is related to his illness. In addition, another question is whether the first manifestations of his disease are interrelated with the rapid weight loss. The fact that the patient’s weight stabilized before disease onset and remained stable ever since, in spite of his gradually evolving muscle weakness and atrophy, is against the view that ALS could account for the weight loss. In addition, an extensive work up ruled out the possibility of the patient’s weight loss. Whatever the causes of the patient’s weight loss are, it is against the view that ALS could account for the weight loss. In addition, an extensive work up ruled out the possibility of the patient’s weight loss. Whatever the causes of the patient’s weight loss are, it is against the view that ALS could account for the weight loss. In addition, an extensive work up ruled out the possibility of the patient’s weight loss. Whatever the causes of the patient’s weight loss are, it is against the view that ALS could account for the weight loss. In addition, an extensive work up ruled out the possibility of the patient’s weight loss. Whatever the causes of the patient’s weight loss are, it is against the view that ALS could account for the weight loss. In addition, an extensive work up ruled out the possibility of the patient’s weight loss.

In the second hair sample of 5 cm length, obtained 3 months after the initial presentation of the patient, represents hair growth of a time period during which his body weight had already been stable for more than 3 months.

Conclusion

Our analytical data derived from the examination of the hair samples, along with self-reported chemical use over decades, provide strong evidence of long term exposure to DDTs and to OPs. In addition, blood levels of the solvents n-hexane and toluene confirm the recent occupational exposure to these substances, which is evidently assured by the patient’s medical report. Based on these findings it can be postulated that in our patient systemic exposure to chemicals may have played a role in MND development.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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