Molecular mechanisms underpinning laser printer and photocopier induced symptoms, including chronic fatigue syndrome and respiratory tract hyperresponsiveness: pharmacological treatment with Cinnamon and Hydrogen

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Abstract

Emissions of laser printers and photocopiers (LP&P) may be associated with health problems. The aim of this review is to describe the clinical picture that is triggered by exposure to LP&P and the molecular mechanisms underpinning the symptoms. Exposure to LP&P to vulnerable subjects may cause a symptom complex consisting of 1) irritation and hyperresponsiveness of the upper and lower respiratory tract; and 2) chronic fatigue (syndrome, CFS). Symptoms occur within hours after L&P exposure and may last for some days or become chronic with exacerbations following LP&P exposure. Substances that can be found in toners or are generated during the printing process are Silica nanoparticles, Titanium Dioxide nanoparticles, Carbon Black, metals, ozone, volatile organic compounds (VOC), etc. The latter may generate oxidative and nitrosative stress (O&NS), damage-associated molecular patterns molecules, pulmonary and systemic inflammation, and modulate Toll Like Receptor 4 (TRL4)-related mechanisms. It is concluded that LP&P emissions may cause activation of the TLR4 Radical Cycle and thus be associated with the onset of chronic inflammatory and O&NS illnesses, such as CFS, in some vulnerable individuals. Cinnamon, an antagonist of the TLR4 complex, and Hydrogen, a potent antiinflammatory and oxygen radical scavenger, may have efficacy treating LP&P-induced illness.

1. INTRODUCTION

Worldwide more than 100 million individuals are daily exposed to emissions of laser printers and photocopiers (LP&P). There is now a vast literature that LP&P emissions may be associated with significant health problems due to the release of nanoparticles and other components in the LP&P emissions (Tang *et al.* 2012). Epidemiological data show that nanoparticle exposure may increase the annual mortality risk with 4–34 additional deaths per million printer users or exposed family mem-

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bers (Hanninen *et al.* 2010). There is also evidence that LP&P emissions and the substances in the emissions may cause immune-inflammatory responses, oxidative and nitrosative stress (O&NS) and cytotoxic and genotoxic effects (Tang *et al.* 2012). For example, in young, healthy, non allergic volunteers a significant increase in pro-inflammatory cytokines, including interleukin-1 (IL-1), IL-6 and IL-8 and tumor necrosis factor (TNF) α, and O&NS biomarkers (DNA damage) was found in urine and nasal lavage 6 hours after exposure to LP&P in a busy photocopy center (Khatri et al. 2012). These elevations in LP&P-induced inflammatory and O&NS biomarkers remained significant for up to 36 hours and indicate that LP&P may induce respiratory tract inflammation in normal volunteers. In animal models it was shown that toner particles may cause lung inflammation with increased levels of pro-inflammatory cytokines, such as IL-1 and IL-6 and O&NS markers, such as nitric oxide synthase (NOS) (Bai *et al.* 2010). The toner particles additionally may enter the alveoli and induce pulmonary lesions and apoptosis and may slow down body weight growth (Bai *et al.* 2010). This pulmonary stress induced by toner particles is long lasting with a clearance period of up to 12 weeks (Bai et al. 2010). Thus, exposure to LP&P may cause chronic pulmonary inflammatory and O&NS responses, which may cause systemic sequels.

Epidemiological studies show that LP&P nanopoarticle exposure not only affects the pulmonary system, but also other organs, e.g. the cardiovasvular system (Bai *et al.* 2010; Nurkiewicz *et al.* 2011). Indoor exposure to volatile organic compounds (VOCs), a complex mixture of organic substances and water in LP&P, is known to cause adverse health effects, including asthma, respiratory lung disease, dizziness and cardiovascular disorders (Guo *et al.* 2009; Bai *et al.* 2010; Nurkiewicz *et al.* 2011). Likewise, animal studies show that exposure to nanoparticles may cause inflammatory and O&NS responses in the lung and consequent changes in heart cells via lung-neuron pathways (Kan *et al.* 2011; Nurkiewicz *et al.* 2011).

It is heavily debated (Tuomi *et al.* 2000) whether LP&P emissions have the potential to cause clinical symptom clusters or disease. For example, the Toner Pilot Study showed that in patients with self-reported hypersensitivity to LP&P emissions "a certain pattern of symptoms is recognizable" (Mersch-Sundermann, 2008). The most important symptoms observed after LP&P exposure are chronic fatigue and airway hyperresponsiveness. However, until now the clinical reactions to LP&P exposure are not well characterized and the pathways underpinning the inflammatory and O&NS responses have remained elusive.

Therefore, the aim of this review is to a) describe the clinical picture that may be triggered by longstanding exposure to LP&P; b) delineate the LP&P substances that have the ability to cause chronic inflammation and O&NS processes; c) describe the molecular mechanisms underpinning these reactions; and d) discuss putative pharmaceutical treatments for these LP&P-induced reactions.

2. CLINICAL PICTURE TRIGGERED BY LP&P EXPOSURE

The spectrum of reported symptoms is very broad. More than 3.000 self-reported cases of LP&P-induced illness are documented in the database of the International Foundation "nano-Control", Hamburg, Germany (https://www.nano-control.de/). A first characteristic are respiratory symptoms, not only acute symptoms such as airway hypersensitivity and asthmatiform reactions in response to LP&P exposure, but also (sub)chronic symptoms, including nose and throat discomfort, burning tongue, chronic rhinitis, cough, and irritation of larynx and vocal chords. Asthma and chronic obstructive pulmonary disease (COPD) are reported to occur in 30% of the cases (personal communication, Stelting, nano-Control). LP&P may cause asthma, vocal chord dysfunction, dysphonia, stridor and chest tightness (Munoz *et al.* 2007). In a 10-year cohort study, toner-exposed workers show a higher prevalence of coughing and sputum and allergic disease (Terunuma *et al.* 2009). Accompanying characteristics are fatigue and exhaustion appearing after inhalation of the emissions, chronically increased fatigue and exhaustion, lack of concentration, word finding problems, depressed mood, dizziness, headaches and sleep disorders, and gastro-intestinal symptoms, reminiscent of irritable bowel syndrome. Fibromyalgic symptoms with pinprick sensation of exposed skin, numbness of hands and feet and headache are often present. Conjunctival irritation, reduced olfaction and hypertension occur frequently. In a later stage, the hypersensitivity is no longer confined to LP&P emissions, but the patients may develop allergic reactions to metals and multiple chemical sensitivity.

In collaboration with the International Foundation "nano-Control" (Hamburg, Germany) a survey was conducted to examine self-reported symptoms following LP&P exposition in thirty six individuals with self-reported chronic LP&P-induced illness. All subjects scored significantly on respiratory symptoms, 91.6% had fatigue or exhaustion, 86.1% suffered from avolition, 72.2% showed neurocognitive symptoms, while 69.4% reported depressed mood. Symptoms may occur within hours after the exposure and may last for some days or become chronic with exacerbations following LP&P exposure. During weekends and holidays the symptoms may improve. All subjects suffered chronically (2–23 years) from this condition.

Thus, two major symptom factors may be detected in these data: 1) hyperresponsivess, hypersensitivity and / or irritability of the upper and lower respiratory tracts, as indicated by chesty cough, asthmatiform reactions, chronic rhinitis, irritation of throat, larynx and

vocal cords, and burning tongue; and 2) symptoms of CF/CFS (and related fibromyalgia) with fatigue, headache, neurocognitive symptoms, inflammatory-like symptoms, gastro-intestinal symptoms, sleep disorders, depressed mood, avolition, hyperalgesia, etc. This symptom complex may be accompanied by conjunctival irritation, reduced olfaction and cardiovascular symptoms, such as hypertension.

3. DETRIMENTAL EFFECTS OF LP&P EMISSIONS

The emissions of LP&P are very complex. It is necessary to distinguish between the toner composition, the emissions and additives. LP&P can be regarded as electro-chemical and thermic reactors, in which massive physical and chemical transformations occur. Hundreds of compositions can be found in patent publications, e.g. in Espacenet, the server of the European Patent Office, searching in the International Patent Classification G03G9/08. However, researchers have almost no access to the composition of toners and the additives used. While the toner contains many putative detrimental substances other chemicals and nanoparticles may be generated during the printing process.

The emission of printers in an indoor environment may significantly increase the level of submicrometer particle numbers in the office and this effect depends upon printer type, numbers of pages printed, cartridge age and indoor ventilation (He *et al.* 2007; Betha *et al.* 2011). A time dependent analysis of the release of particles shows that at the beginning of the printing process tone powder nanoparticles are set free with high levels of Ba, Zn, B, K, Sr and Na, whereas in later phases of printing larger nanoparticles are observed due to condensation of vapors (Castellano *et al.* 2012). Higher sized particles are released when printing on paper. Not only the toner but also the high-temperature fuser unit is a source for nanoparticle emission (Wensing *et al.* 2008). Some particles are released in bursts, whereas others are continuously released during printing (Schripp *et al.* 2008). The toner consists of toner particles with a typically average size of 2 μm to 10 μm (Wensing *et al.* 2010; 2011). Toner particles are composed of pigments, polymers and/or wax and are melted onto paper in the fuse unit of the printer during the printing process. Most toners comprise several free solid nano-additives, which are used as charge control agents and to tune the rheological properties of the toner. These are mostly chemical modified Silica nanoparticles or Titanium Dioxide nanoparticles. They can be identified in the material safety data sheets of the toners as Titanium Oxide (CAS No. 13463-67-7) and Silica (CAS No. 7631-86-9). The diameter of a typical nanoparticle in toner is in the range of 6 nm to < 100 nm.

3.1. Metals

Many toners comprise metals. Many patients with LP&P-induced illness also show allergies to metals. Thus, of the 28 individuals sensitive to LP&P emissions, 54% reacted in an epicutane test to nickel, 25% to cobalt and 21.4% to mercury (Palm, 2006). Magnetite (iron (II,III) oxide, Fe2+Fe3+2O4) is often used as black pigment, e.g. Magnetite 30% - 40% Weight (Kyocera Mita, 2009). Iron is the central atom of hemoglobin and therefore essential for humans, but iron in oxidized form, as can be found in Magnetite, may cause adverse health effects. In rodent studies, subchronic inhalation of Magnetite (Fe(3) O(4)) for 6 weeks resulted in elevated numbers of neutrophils in bronchoalveolar lavage (BAL) and caused histopathological changes in the upper respiratory tract and nasal passages (Pauluhn, 2012). Higher Magnetite concentrations may cause pulmonary inflammation and increased collagenous fibers and lymph node weights (Pauluhn, 2012). In another study, dramatic effects were detected after short term high level exposition to Magnetite nanoparticles (Srinivas *et al.* 2012). Rats showed reduced cell viability in the lung, significantly higher levels of pro-inflammatory cytokines and malodialdehyde (MDA), reduced antioxidant enzyme activities and structural alterations.

Beside iron, other metals are found in toners, e.g. titanium, chromium, nickel, zinc, aluminum, copper, cobalt, and zirconium (JP2001272823A; JP2009042447A JP2009282350A; Barthel *et al.* 2011). Inspecting the patent literature, it appears that these heavy metals are not impurities, but that they are added intentionally to the toners. For example, the European Patent Toner for developing electrostatic image (EP 0662638 B1) lists the following as potential compounds of the toner: "triiron tetroxide (Fe3O4), diiron trioxide (γ-Fe2O3), zinc iron oxide (ZnFe2O4), yttrium iron oxide (Y3Fe5O12), cadmium iron oxide (CdFe2O4), gadolinium iron oxide (Gd3Fe5O12), copper iron oxide (CuFe2O4), lead iron oxide (PbFe12O19), nickel iron oxide (NiFe2O4), neodymium iron oxide (NdFe2O3), barium iron oxide (BaFe12O19), magnesium iron oxide (MgFe2O4), manganese iron oxide (MnFe2O4), lanthanum iron oxide (LaFeO3), powdery iron (Fe), powdery cobalt (Co), and powdery nickel (Ni)". Metal compounds are known to be carcinogens, cause an increased generation of free chemical radicals (Koedrith and Seo, 2011), and induce oxidative stress and DNA repair deficiencies (Koedrith and Seo, 2011). In humans, nickel may cause contact dermatitis, allergic contact urticaria, rhinitis and asthma (Estlander *et al.* 1993). Schmidt *et al.* (2010) provided evidence that the Toll Like Receptor (TLR)4 complex plays a role in the mechanisms leading to contact nickel allergy, suggesting that also the innate immune system can substantially contribute to "allergic" reactions. Type IV hypersensitivity reactions are routinely tested with a patch test although in cases of metal hypersensitivity,

the Memory Lymphocyte Immuno Stimulation Assay (MELISA), a modified lymphocyte transformation test (LTT), may be superior (Valentine-Thon *et al.* 2007).

3.2. Solid Nanoparticles

The LP&P emissions can be regarded as a major source of volatile and solid nanoparticles, whereby many different solid nanoparticles are used for different purposes. Between 0.2% to 1.9% of the emissions of laser printer are solid inorganic compounds (Barthel *et al.* 2011). During a single print job, up to $1.6\ 10^9$ to $1.5\ 10^{10}$ solid particles may be measured (Barthel *et al.* 2011). These findings indicate that solid nanoparticles are set free during printing processes.

3.2.1. Carbon Black

Magnetite or Carbon Black are frequently employed as black pigments. For example, according to the material safety data sheet the Toner TK-17 comprises: "Styrene Acrylate Copolymer 50%–60%; Magnetite 30%–40%; Titanium Oxide (CAS No. 13463-67-7) 1%–5 %; Silica (CAS No. 7631-86-9) 1%–5% and Carbon Black (CAS No. 1333-86-4) < 1%" (Kyocera Mita, 2009). Between 2% and 11% of the weight of this toner consist of solid nanoparticles. Due to their small size, their numbers are extremely high. The biological relevant surface even of 1% nanoparticles (mass) can be a factor 10 larger that the whole surface of the 99% (mass) toner particles. In contrast to toner particles, inhaled nanoparticles can enter the blood stream easily. The nanoparticles first bind to the proteins of the epithelial lung lining fluid and the consequent formation of conjugates of nanoparticles with these proteins and thus their hydrophobic character determines the translocation of the nanoparticles through the air-blood-barrier (Fertsch-Gapp *et al.* 2011). The uptake of nanoparticles causes the release of inflammatory mediators and cell death. For example, Carbon Black induces cell death by pyroptosis (Reisetter *et al.* 2011). In combination with the intratracheal administration of bleomycin, Carbon Black nanoparticles show synergistic effects (Kamata *et al.* 2011) and causes fibrotic changes and pulmonary inflammatory infiltrations with enhanced IL-6 and keratinocyte chemoattractant (Kamata *et al.* 2011). Moreover, Carbon Black nanoparticles are known to be carcinogenic, a mechanism that is presumably based on the binding to arylamine N-acetyltransferase (Sanfins *et al.* 2011). Carbon Black nanoparticles are known to impair the detoxification pathways, while pulmonary exposure to Carbon Black nanoparticles is a risk factor for atherosclerosis and vasomotor dysfunction (Muller *et al.* 2011). Yamamoto et al. (2006) found that in mice, Carbon Black nanoparticles synergistically augmented the inflammatory properies of staphylococcal lipoteichoic acid.

In another study the effects of four different nanoparticles were examined, i.e. Carbon Black, nickel, cobalt and titanium dioxide (Dick *et al.* 2003). Instillation with Carbon Black and ultrafine cobalt caused a massive influx of neutrophils and an increase in inflammatory markers, e.g. macrophage inflammatory protein-2 (Dick *et al.* 2003). The inflammatory reaction to ultrafine nickel appeared to be delayed, but ultimately resulted in a comparable response as that observed for Carbon Black and ultrafine cobalt. In contrast, ultrafine titanium dioxide did not cause significant increases in neutrophils (Dick *et al.* 2003). Ultrafine cobalt, nickel and Carbon Black not only cause a significant release of inflammatory markers, but also free radicals and reactive oxygen species. Some but not all nanoparticles may cause adverse effects in individuals affected by COPD or asthma through activation of inflammatory and oxidative stress pathways (Dick *et al.* 2003). Nevertheless, Carbon Black is encapsulated in the toner particles consisting of wax and other polymers. Therefore, a massive release of Carbon Black nanoparticles into the air cannot be assumed since most Carbon Black, but not necessary all, will be captured within the toner particles when they are melted onto the paper.

3.2.2. Charge Control Additives

In contrast to Carbon Black, which is encapsulated in the toner particles, diverse solid nanoparticles are added as charge control agents. They are used to fine tune the electrostatic properties of the toner (Winkelmann and Lutz, 2011; www.printers2day.com). In a first step, the drum of the laser printer or photocopier is electrostatically charged by high voltage (600 V to 2.000 V). Consequently, the document is exposed and scanned and the light reflected on the drum extincts the charge. In a next step, the toner particles are electrostatically transferred to the drum and the toner transferred to paper, which is more charged that the latent image on the drum. In the fuse unit of the printer, the toner is melted onto the paper at high temperature. Silica and titanium dioxide nanoparticles are most often used as additives. They are chemically modified, e.g. salinized with positive or negative charged groups (DE102006053160A1). The linkage between the silane-groups and these nanoparticle is often obtained with halogens, like chlorine, bromine and iodine (DE19929845A1). For example, in the European patent application form KYOCERA MITA CORP [JP] EP1246023A2 (2002) it is stated: "[0006] As the aforementioned charge control agent, a metal complex or nigrosine dye is used. Many of such substances, however, contain a heavy metal or aniline, and therefore their use may be restricted if the guidelines for their safety are revised to be stricter in the future. The same may happen also with carbon black used as a colorant. ...". The author of this patent application was apparently aware of the putative adverse effects of the ingredients used. The anticipated more restrictive guidelines, however, are not applied and as a result Carbon Black and heavy metals are still standard in toners.

Titanium dioxide (TiO2) nanoparticles may cause irritations of the respiratory tract, one of the symptoms of LP&P-induced symptom cluster. TiO2 nanoparticles can induce mucin secretion resulting from increased intracellular Ca(2+) concentrations, suggesting that exposure to some nanoparticles may play a role in the onset of asthma and COPD (Chen *et al.* 2011). Moreover, maternal exposure to TiO2 nanoparticles during pregnancy has multiple effects on newborn rats, including altered expression of genes associated with apoptosis, brain development, oxidative stress and neurotransmitters, mechanisms that play a role in brain disorders, such as Alzheimer Disease, Attention Deficit Disorder, Autism, Parkinson Disease, Epilepsy and Schizophrenia (Shimizu *et al.* 2009). The intranasal exposure to TiO2 nanoparticles during pregnancy causes an increased susceptibility to asthma in the offspring (Fedulov *et al.* 2008), suggesting that LP&P emissions should be taken into account as a contributing factor to the rising incidence of asthma (WHO, 2011).

Epidemiological studies show that pulmonary TiO2 nanoparticle exposure is associated with cardiovascular morbidity (Nurkiewicz *et al.* 2011). The inhalation of TiO2 nanoparticles may impair vascular function and may play a role in atherosclerosis (Kan *et al.* 2011). Pulmonary exposure to TiO2 nanoparticles may alter the phosphorylation patterns of cardiac proteins, whereas direct incubation of isolated cardiac myocytes with TiO2 nanoparticles did not alter the phosphorylation status of proteins and did not result in systemic inflammation (Kan *et al.* 2011). The authors conclude, that a lung-neuron-regulated pathway may be involved, e.g. increased levels of substance P levels in the heart following TiO2 nanoparticle exposition of the lung (Kan *et al.* 2011). A study examining the effects of TiO2 nanoparticle inhalation on systemic microcirculation found altered response of smooth muscles to NO, a decreased availability of NO associated with arteriolar dysfunction, a significantly changed signature of pro-inflammatory cytokines and increased O&NS (Nurkiewicz *et al.* 2009). It was concluded that TiO2 nanoparticles cause vascular effects, which are attributable at least in part to inflammatory and O&NS pathways.

Finally, nasally instilled TiO2 nanoparticles are translocated into the brain and induce morphological changes in the brain, primary in the hippocampus where high Ti contents are found (Wang *et al.* 2008). The TiO2 nanoparticles induce oxidative stress and lead to the release of high amounts of NO and glutamic acid (Wang *et al.* 2008). Incubation of mouse microglia with TiO2 nanoparticles causes an acute and long-standing release of ROS (Long *et al.* 2006). Even low concentrations of TiO2 nanoparticles rapidly damage complex brain cultures, presumably through the effects of increased ROS (Long *et al.* 2006).

3.3. Ozone

A comparison of charged aerosol in 10 copy centers revealed much higher (up to 19.5 times) ion concentrations in the air of the copy centers than in the control site (Han *et al.* 2011). This effect may result from charge control agent nanoparticles, high voltage discharge ionization and ozone. Due to high voltage discharge LP&P produce ozone, a molecule with three oxygen atoms that is highly reactive. Ozone can oxidize many organic molecules, including proteins and lipids, and is classified as being carcinogenic. Increased exposure to ozone is accompanied by increased pulmonary-related mortality and morbidity (Hollingsworth *et al.* 2007) and asthma exacerbations (Peden, 2011). The "Berufsgenossenschaft ETEM" concluded that ozone, if located directly in the airflow of the laser printer, can have detrimental effects in some vulnerable individuals (BG-Infoblatt, 2010).

In animal models, pre-exposure to ozone causes increased airway hyperreactivity and elevated pro-inflammatory cytokine levels in lung lavage fluid, increased LPS-mediated signaling in lung tissue, and changes in the distribution of macrophage TLR4 (Hollingsworth *et al.* 2007). These findings show that ozone pre-exposure enhances the pulmonary response to inhaled LPS by priming the innate immune system (Hollingsworth *et al.* 2007; Al-Hegelan *et al.* 2011). While it is known that inhalation of ozone induces sterile inflammatory responses and tissue injury, TLR4 deficient mice lack these ozone-induced responses (Connor, 2012). Other studies show that ozone-induced airway hyperresponsiveness depends on the presence of functional TLR2 and TLR4, and MyD88 as well (Williams *et al.* 2007). Similar findings were reported by Li et al. (2011) who found that ozone-induced airway hyperresponsivess depends on intact TLR4, MyD88 (an intracellular messenger molecule of the TLR4 complex) and TIRAP (Toll-interleukin 1 receptor domain containing adaptor protein) (Li *et al.* 2011). Mice deficient in these genes show reduced response to challenge with ozone or hyaluronan (Li *et al.* 2011). Garantziotis et al. (2009; 2010) reported that ozone-induced airway hyperresponsivess is partly mediated by extracellular matrix hyaluronan and the TLR4 through increased production of pro-inflammatory cytokines and modifying the biological responses to hyaluronan. Bauer *et al.* (2011) examined the effector molecules downstream of the TLR4 complex and delineated heat-shock protein Hsp70 contributes to ozone-induced inflammation in the lungs.

All in all, ozone has similar characteristics as LPS, such as increased responses to allergens, neutrophilic inflammation, pro-inflammatory cytokines and O&NS processes and TLR4 and inflammasome signaling (Peden, 2011).

3.4. Volatile Organic Compounds (volatile nanoparticles)

The major fraction of the nano-particular LP&P emissions consist of volatile organic compounds (VOCs) (Barthel *et al.* 2011). These VOCs are condensation products of organic substances and water which result from the partial vaporization of toner and paper in the fuse unit of LP&P at a temperature of approximately 170°C. The Toner Pilot Study (Mersch-Sundermann, 2008) described different substances in laser printer VOCs, such as methylcyclohexane, benzene, toluene, ethylbenzene, n-propylbenzene, meta-xylene, para-xylene, 1,3,5-trimethylbenzene, styrene, (+)-α-pinene, delta-3-carene, limonene, benzaldehyde and acetophenone. The average concentrations of these putative carcinogenic substances was in the range of 1 µm/m3 to 30 µm/m3 with maxima of more than 280 µm/m3 (Mersch-Sundermann, 2008). Ceraceous paraffin wax carbohydrates and organosilicons are other volatile nanoparticles that can be detected in LP&P emissions (Wensing *et al.* 2011). The numbers of VOCs during printing process can be extraordinary high. Typical values during normal office operations are about 10.000 VOC / cm^3 (Mohlmann 2005), but values as high as 4×10^5 and 1×10^8 VOCs / cm^3 are reported (Martin *et al.* 2011; Lee and Hsu, 2007).

The US Environmental Protection Agency listed the following potential health effects of VOCs: "Eye, nose, and throat irritation, headaches, loss of coordination, nausea, damage to liver, kidney, and central nervous system. Some organics can cause cancer in animals; some are suspected or known to cause cancer in humans. Key signs or symptoms associated with exposure to VOCs include conjunctival irritation, nose and throat discomfort, headache, allergic skin reaction, dyspnea, declines in serum cholinesterase levels, nausea, emesis, epistaxis, fatigue, dizziness" (EPA, 2013).

In elderly persons, exposure to VOCs, such as toluene and styrene, exert detrimental effects on pulmonary functions (as measured by spirometric tests) by increasing O&NS processes (i.e. biomarkers of damage to DNA and lipid peroxidation) (Yoon *et al.* 2010). Some VOCs, such as toluene, benzene and styrene, cause an increased production of inflammatory mediators, such as prostaglandins and cyclo-oxygenase 2, in the human lung epithelial cells (Mogel *et al.* 2011). In animal studies the exposure to VOCs causes airway inflammatory responses, significantly enhanced IL-6 concentration and O&NS processes including increased NOS activity (Wang *et al.* 2012). VOC exposure may additionally modulate neurological signaling of airway inflammatory response via the NO signaling pathways (Wang *et al.* 2012).

The fraction of air comprising nanoparticles is often termed particulate matter (PM) 2.5, i.e. the standard fraction of particles smaller than 2.5 µm. Long-term subacute exposition with PM2.5 causes significant cardiovascular events in human (Miller *et al.* 2007; Puett *et al.* 2008). In healthy subjects, PM2.5 may cause airway inflammation (Schaumann *et al.* 2004). PM2.5 exposure causes inflammation and plaque formation in the cardiovascular system and is thus associated with atherosclerosis, myocard infarct and increased mortality (Pope *et al.* 2006; Nawrot *et al.* 2011). In a mouse model, chronic exposition to PM2.5 leads to formation of ROS with involvement of NADPH oxidase, whereas in TLR4 and Nox2 deficient mice no such reactions are established (Kampfrath *et al.* 2011). Exposure to PM2.5 increases oxidized phospholipid derivatives of 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine (oxPAPC) in bronchioalveolar lavage (Kampfrath *et al.* 2011).

VOCs become even more hazardous to health, when they come in contact with ozone. Ozone usually reacts very fast with most materials and is then eliminated. The reaction of ozone with VOCs generates long-lived reactive oxygen intermediates (ROIs) which may be stable for minutes (Shiraiwa *et al.* 2011). The implications are that the allergic and toxic effects of VOCs are increased by the presence of ozone (Shiraiwa *et al.* 2011). Moreover, the ozone concentrations generated during the printing process may be systematically underestimated because due to the high VOCs the concentrations of ozone will decrease to form ROIs.

4. MOLECULAR MECHANISMS UNDERPINNING THE INFLAMMATORY AND O&NS RESPONSES TO LP&P

4.1. Role of the TLR Radical Cycle

In the previous section we have described that different constituents of LP&P emissions may cause (sub) chronic inflammatory and O&NS processes. Therefore, we propose to term this condition LP&P-induced Chronic Inflammation (LICI). Moreover, the results of different studies described in the previous section show that the TLR4 complex may be a key phenomenon underpinning LICI.

TLRs play a crucial role in the fast detection and defense against viruses, bacteria and fungi. In contrast to the adaptive immune system which comprises antibodies and T-Cell receptors, the innate immunity reacts without any delay. Humans have ten different functional TLRs, i.e. TLR1 to TLR10. Beside TLR4 also TLR2 plays an important role in many diseases of civilization, like asthma, COPD, stroke, myocardial infarction, arteriosclerosis, diabetes, cancer, liver cirrhosis and many more (Lucas and Maes, 2013). TLRs recognize and are activated by either pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). The major PAMPs are bacterial lipopolysaccharides (LPS). Typical DAMPs, which act as agonists for TLR4, are hyaloron fragments, oxidized phospholipids, fibronectin extra domain A, heat shock protein 70 (HSP70), substance P and activated HMGB1 (Lucas and Maes, 2013).

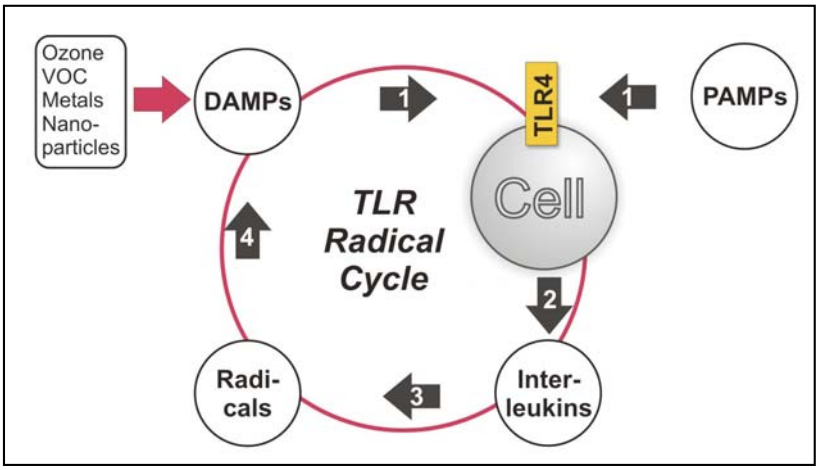

Figure 1. The Toll Like Receptor (TLR)4 Radical Cycle.

As explained in the previous section, many constituents of LP&P emissions cause increased ROS, which subsequently oxidize lipids and proteins or degrade structural molecules thereby forming DAMPs, such as hyaloron, oxidized phospholipids, which in turn will activate the TLR4 Radical Cycle (Lucas and Maes, 2013). For example, TiO2 nanoparticle exposition of the lung may affect heart functions through induction of substance P, one of the DAMPs, which functions as a TLR4 agonist (Kan *et al.* 2012). TiO2 nanoparticles additionally trigger transgenic cell-lines expressing human TLR4, showing that the adverse effects of TiO2 nanoparticles may be associated with their effects on the innate immune system (Chen *et al.* 2011). Ozone, another LP&P constituent, displays similar characteristics as LPS, and induces inflammatory and O&NS processes through TLR4 signaling involving heat-shock protein Hsp70, another DAMP (Peden 2011; Bauer *et al.* 2011]. Moreover, Bauer et al. (2011) showed that MyD88-dependent and independent pathways participate in ozone-induced TLR4 signaling. Exposure to PM2.5 induces the formation of oxidized phospholipid derivatives of oxPAPC, another DAMP (Kampfrath *et al.* 2011). Carbon Black nanoparticles and staphylococcal lipoteichoic acid induce TLR2-mRNA expression and the levels of pro-inflammatory indicators (Yamamoto *et al.* 2006). The metals in LP&P emissions may interact with the TLR4 complex. Thus, in a recent published paper it was shown that nickel and cobalt can induce TLR4 homodimerization and activation without involvement of the TLR4 accessory protein MD2 (Raghavan *et al.* 2012).

Thus, different substances in LP&P emissions may share a common molecular mechanism that causes chronic inflammation and O&NS, i.e. activation of the TLR4 Radical Cycle (Lucas and Maes, 2013). **Figure 1** shows the different phases in this vicious cycle between TLR4 activation and the formation of DAMPs. In brief, a number of substances and nanoparticles released during the printing process induce O&S damage and chronic inflammation in the lungs and other organs, such as brain and cardiovascular system. These processes may lead to the formation or secretion of DAMPs, including oxidized phospholipids, heat shock proteins and substance P, which all act as TLR4 agonists and therefore may activate the TLR4 complex thus driving the TLR4 Radical Cycle. Moreover, prolonged activation of TLR4 and TLR2 by ozone, TiO2 nanoparticles, silver nanoparticles and toluene, but also DAMPs, such as Substance P, increases expression of these receptors or MyD88 (Williams *et al.* 2007; Cui *et al.* 2001; Win-Shwe *et al.* 2011; Tancowny *et al.* 2010). There is a cross-reactivity between different TLR4 agonists. Thus, prior activation of the TLR may induce greater inflammatory responses by macrophages following activation by another TLR agonist (Pestka and Zhou, 2006).

It may be hypothesized that some individuals are more vulnerable to the detrimental effects of LP&P emissions, e.g. those with pre-existing dysfunctions in the TLR4 Radical Cycle (Lucas and Maes, 2013) or those with an increased LPS load through prior lung inflammation (Hussain *et al.* 2013) or increased translocation of gram negative commensal bacteria (Maes *et al.* 2008). In such a scenario, LPS from gram negative bacteria, e.g. through "leaky gut", could prime TLRs to be sensitized to stimulation by DAMPs. Another putative vulnerability factor is psychological stress. Different type of stressors, e.g. social stress, may increase TLR expression on splenic macrophages (Bailey *et al.* 2007). Transgenic mice lacking TLR4 perform better and display less central immune-inflammatory and O&NS reactions to immobilization stress that mice with normal TLR4 expression (Caso *et al.* 2008). Interestingly, psychological stress may also sensitize TLR to subsequent activation by DAMPs (Lewthwaite *et al.* 2002; Garcia-Bueno *et al.* 2008). In addition, psychosocial stress induces the production of pro-inflammatory cytokines, e.g. IL-1, IL-6, TNFα and interferon (IFN) γ (Maes *et al.* 1998; Steptoe *et al.* 2001) and O&NS damage to DNA and lipids (Pertsov *et al.* 1995; Sosnovskii and Kozlov, 1992; Sivonova *et al.* 2004; Irie *et al.* 2001).

4.2. Mechanistic explanations of LP&P-induced symptoms

As described in section 2, LP&P emissions may induce a symptom cluster consisting of two major factors, i.e. 1) hyperresponsivity or irritability of the upper and lower respiratory tract; and 2) chronic fatigue (syndrome). These two symptom clusters may be accompanied by cardiovascular symptoms. LP&P may thus cause a multisystem condition or illness affecting the lungs, brain function and immune, nervous and cardiovascular system. Exposure to nanoparticles, such as VOCs, most often occurs by inhalation but occasionally also dermal and ingestion exposure may occur (Jakubowski and Czerczak, 2009). The involvement of many organs following inhalation exposure may be explained by the knowledge that not only lung tissues are affected by LP&P emissions, but also other organs, like heart and brain. Substances, such as ozone, ROI-charged VOCs and metals, may additionally enter the blood stream via the lung (Connor *et al.* 2012; Peden, 2011; Williams *et al.* 2007; Ganguly *et al.* 2011; Fedulov *et al.* 2008), inducing inflammatory and O&NS processes and forming DAMPs in target organs. Ozone and VOCs may react instantly with any tissue (Williams *et al.* 2007; Bonish *et al.* 2012) and generate DAMPs, which can be distributed by the blood stream, while metals and nanoparticles can be transported by the blood into other tissues, including the brain (Gustafsson *et al.* 2011; Luo *et al.* 2009).

We now will discuss the mechanistic explanations underpinning the onset of the symptoms that characterize LP&P-induced illness.

1) Airway hypersensitivity and other impairments of the respiratory tract, including asthma exacerbations, cough, chronic rhinitis, burning eyes, throat and vocal cord discomfort, burning tongue are readily explained by the effects of for example Magnetite, TiO2 nanoparticles, ozone and VOCs inducing increased responses to allergens, increased mucin secretion, local (neutrophilic) inflammation and inflammasome signaling, O&NS processes, local lung lesions, etc (Pauluhn 2012; Peden, 2011; Chen *et al.* 2011; Yoon *et al.* 2010; Mogel *et al.* 2011).

2) There is now a vast literature that CFS is accompanied by a complex interplay between activated immune-inflammatory (including translocation of gram negative commensal bacteria) and O&NS pathways leading to autoimmune reactions, mitochondrial dysfunctions and brain disorders (Maes and Twisk, 2010; Morris *et al.* 2013). In those papers, evidence was provided that these pathways may contribute to the onset of specific CFS symptoms, such as chronic fatigue, exhaustion, irritable bowel syndrome, neurocognitive disorders, infectious or inflammatory symptoms (e.g. a flu-like malaise), and fibromyalgic symptoms and hyperalgesia. The latter may also be associated with activation of the TLR4 Radical Cycle. Thus, in an animal model, administration of a TLR4 antagonist, LPS-RS, blocked the onset of allodynia by glucuronic acid and ethyl glucuronide (Lewis *et al.* 2013). In another study, patients with chronic pain show an increased responsiveness of peripheral blood mononuclear cells to TLR agonists (i.e. TLR2, TLR4 and TLR7) than controls without pain (Kwok *et al.* 2012). As such, CF/CFS may be secondary to pulmonary inflammatory, O&NS and histopathological changes that are expressed in the upper and lower respiratory tract and eventually also in peripheral blood, heart and brain.

3) Many patients affected by LICI also show cardiovascular symptoms, including a high blood pressure. As described in section 3, pulmonary exposure to some of the compounds, e.g. TiO2 nanoparticles, is associated with increased cardiovascular morbidity and specific alterations in cardiac myocytes (Nurkiewicz *et al.* 2011; Kan *et al.* 2012). Moreover, there is now epidemiological evidence that inflammatory and O&NS processes are associated with an elevated blood pressure and that the mechanisms comprise platelet activation, thrombogenesis, increased vasoconstriction and direct effects of inflammatory and O&NS markers, including CRP, haptoglobin, IL-6, LPS, nuclear factor-(NF) κB, etc. (Ghanem and Movahed, 2007; Maes *et al.* 2011). As explained in section 2 many patients with LP&P illness react to metals, such as nickel, cobalt and mercury (Palm, 2006). Metals are known to cause O&NS and inflammatory processes (Koedrith and Seo, 2011; Tinkov *et al.* 2012; Checconi *et al.* 2013; Pereira *et al.* 2012) and some metals may activate the TLR4 complex (Raghavan *et al.* 2012).

5. TREATMENTS

5.1. General

As discussed previously (Lucas and Maes, 2013) there are three approaches to block the consequences of an activated TLR4 Radical Cycle. 1) Strategies which neutralize LPS, e.g. using synthetic anti-LPS peptides (SALPs), purified recombinant Factor C), etc. 2) Antagonism of the TLR4 complex, e.g. by employing cyanobacterial product (CyP), E5531, E5564, epigallocatechin-3-gallate, licorice, Magnolia officinalis, Ginger (Zingiberofficinale), Salvia miltiorrhiza (Red sage), Curcumin and Cinnamon extract, which suppresses the induced overexpression of MyD88. 3) Antiinflammatory and antioxidative strategies, e.g. N-acetyl cysteine, molecular Hydrogen, etc. (Lucas and Maes, 2013).

In section 6 we present a case report on a patient with LP&P-induced airway hyperresponsiveness and CFS. This patient was successfully treated with compounds that target the immuno-inflammatory and O&NS pathways, i.e. Cinnamon and Hydrogen, suggesting that these pathways underpin the pathophysiology of LP&P-associated CFS. Before describing this case report we will describe the effects of the compounds on immuno-inflammatory pathways, including the TLR4 radical cycle.

5.2. Cinnamon.

Cinnanmon extract has significant antiinflammatory and antioxidant properties (Kumar *et al.* 2012; Yang *et al.* 2012; Dhuley, 1999; Ho *et al.* 2013). For example, anti-inflammatory effects are shown for Myristicin (1-allyl-5-methoxy-3,4-methylenedioxybenzene) one ingredient in Cinnamon (Lee and Park, 2011]. Cinnamon extract has a significant effect on the TLR complex and is an antagonist of MyD88. In vitro studies show that Cinnamon extract suppresses LPS-induced MyD88 and iNOS, and TNFα expression and NO synthesis (Kanuri *et al.* 2009). In mice, Cinnamon extract may block alcohol-induced steatosis in association with attenuation of MyD88 mRNA, iNOS and plasminogen activator inhibitor 1 expression. Cinnamaldehyde, another active ingredient of cinnamon, inhibits the oligomerization of TLR4 (Youn *et al.* 2008) and is therefore a TLR4 antagonist. Similar molecular TLR4-antagonistic properties are described for Curcumin (from Turmeric or Curcuma longa) (Youn *et al.* 2006) and 6-shogaol (Ahn *et al.* 2009) an active ingredient of ginger (Zingiber officinale).

Cinnamon gains growing attention in several diseases which are associated with activation of the TLR Radical Cycle. For example, several studies emphasize the beneficial effects of Cinnamon for type 2 diabetes mellitus (Jiao *et al.* 2013; Qin *et al.* 2010; 2012). Glucose, lipid metabolism and inflammation all improve during treatment with cinnamon in humans, animal models and cell culture models as well (Qin *et al.* 2012). Cinnamon may have beneficial effects in metabolic syndrome (Cao *et al.* 2008) and cardiovascular diseases (Qin *et al.* 2010). In addition, Cinnamon extract is shown to induce tumor cell death by inhibition of NF-kB and AP1 in various cancer cell-lines, including cervix cancer, colorectal cancer, lymphoma, melanoma and in a vivo in mouse melanoma model (Kwon *et al.* 2010).

5.3. Molecular hydrogen

Molecular hydrogen (H2) is the smallest of all molecules and a radical scavenger with specific properties. Molecular hydrogen is inert indicating that it does not react with non-radical molecules or ions. H2 does react at a slow rate with oxide radical ions and hydroxyl radicals (Ohno *et al.* 2012). A problem of almost all pharmaceuticals is the delivery of the substance to the location where it should deploy its effect. The diffusion properties of hydrogen, however, are exceptional. Hydrogen can cross membranes and gets into cytoplasm and even into mitochondria. The blood brain barrier (BBB) does not hinder hydrogen from entering the brain. As discussed previously, molecular hydrogen has anti-inflammatory, anti-oxidative and neuroprotective effects (Lucas and Maes, 2013). Interestingly, hydrogen gas blocks LPS-induced production of NF-κB and activation of inflammatory pathways (Xie *et al.* 2012). Therefore hydrogen has several properties, suggesting that it can be used as an antioxidant and anti-inflammatory agent and by inference that it could be used as a new drug for the treatment of inflammation and O&NS-related disorders, such as CFS.

A recent review argued that Hydrogen may have a clinical efficacy in human diseases, such as myocard infaction, diabetes mellitus type 2, metabolic syndrome, etc. (Ohno *et al.* 2012). Animal models showed that hydrogen may be useful in the treatment of neuroinflammatory disorders, such as Alzheimer's and Parkinson's disease models (Ohno *et al.* 2012). Acute side effects after short time application of hydrogen are not to be expected. Thus, professional divers who dive deeper than 100 m cannot breathe the compressed air because of the properties of nitrogen. Therefore they often use HydroxTM, a mixture of hydrogen and oxygen, or HydrelioxTM, a mixture of hydrogen, helium and oxygen. These gas mixtures contain up to 49% Hydrogen. At depths deeper than 300 meters a phenomenon termed hydrogen narcosis may become relevant (Larry Harris Taylor, 2004). No side effects were observed at pressure up to 10 bar (1 Mpa) (personal communication Bernard Gardette, COMEX, France). One potential risk of hydrogen would be a reaction with nitric oxide (NO), which regulates the dilation of blood vessels and acts as a neurotransmitter (Murad, 2004). Ohsawa et al. (2007), however, showed that hydrogen does not react with NO.

6. CASE REPORT OF A PATIENT WITH LP&P-INDUCED CFS.

XX, a man of 52 years of age, was affected by a syndrome induced by LP&P emissions. XX was working in an industrial office. Begin January 2010 all office devices of the company, including laser printers, were changed. Within 10 weeks XX developed a hypersensitivity to the emissions of the newly installed LP&P. Mid March 2010, XX developed flu-like symptoms and a profound fatigue appeared. Moreover, the mucosa of the nose and tongue were swollen and irritation of vocal chords and larynx further impaired the articulation of words. Within minutes after contact with the LP&P emissions sensations of burning tongue appeared. Running nose and chesty cough became chronic. Other symptoms were: chronic fatigue and exhaustion, headache, dizziness, word finding difficulties combined with mild depression, muscle weakness and aches. XX also developed high blood pressure, around 160–170 mm/Hg, although the diastolic value was never higher than 100–110 mm/Hg.

After a major print job in March 2010, acute asthmatiform reaction forced XX to leave the office room. In June 2010, hypersensitivity was at a maximum and exposure to LP&P emissions for some minutes resulted in profound exhaustion which could last for 3 days. From this point XX avoided any exposure to LP&P devices and in July 2010 XX had to quit his job. The

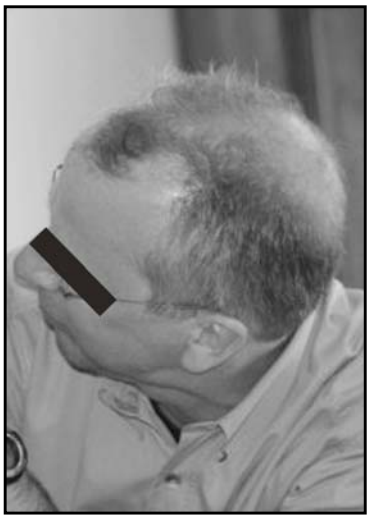

Figure 2A. Patient XX, massive hair loss during the hypersensitive phase to laser printer emissions. Picture was taken in July 2011.

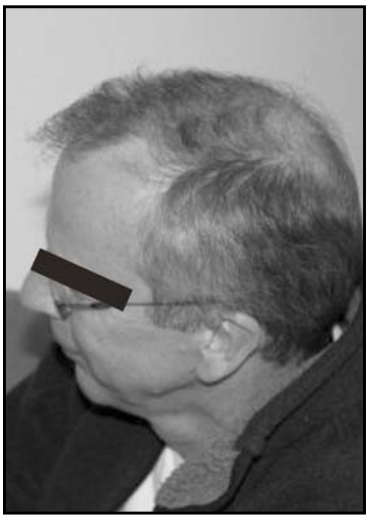

Figure 2 B. Patient XX in June 2012, ten month after starting Hydrogen therapy.

hypersensitivity and chronic fatigue were accompanied by massive hair loss (see **Figure 2a**). The hypersensitivity was very specific to LP&P emissions, with two exceptions: grinding iron and hardware stores could also cause hypersensitivity reactions starting with burning tongue sensations. The reaction to air in hardware stores may be explained by effects of indoor metal vapors and metal allergic reactions.

July 2010 the chronic fatigue was extreme: XX could only exert very mild physical activity for maximal 4 hours / day, while the 20 remaining hours of the day had to rest. At that time, the patient complied with the diagnostic CDC criteria for CFS (Fukuda *et al.* 1994), i.e. he suffered from disabling CF for more than 6 months and from more than four typical CFS symptoms, i.e substantial impairment in short-term memory and concentration; sore throat that is frequent or recurring; headache of new type; unrefreshing sleep; and post exertion malaise lasting more than 24 hours.

Fourteen months of nearly total abstention from LP&P emissions only slightly improved the hypersensitivity reactions. August 2011, XX started an experimental treatment with Cinnamon extract (Tinctura Cinnanomi 1:5, Maros Arznei GmbH) 4 mL diluted in a glass of water per day. One week after starting this treatment the hypersensitivity improved and after three weeks treatment the hypersensitivity had completely resolved. Even after exposure to two laser printers in a room of 12 square meters for more than 30 minutes no hypersensitivity reaction emerged. Although treatment with Cinnamon extract was discontinued 2 weeks later, the effects of Cinnamon extract blocking the hypersensitivity reactions lasted until the data of submission of this paper. Also, during treatment with Cinnamon, the lost hair gradually grow back and by June 2012, ten months after starting Cinnamon hair growth was partially re-established (**see Figure 2B**).

Despite the progress made, the abovementioned CFS symptoms were still present and therefore XX started a new treatment in June 2012 with another radical scavenger, i.e. Hydrogen. Because Hydrogen enriched water is not commercially available, a proprietary method was used (German patent application filed). The solubility of Hydrogen in water is approximately 0.8 mMol. Therefore only milligram amounts of substance is needed to obtain the maximal soluble Hydrogen for one liter of water. Water should be deionized because, even if Hydrogen is inert, during the reaction Hydrogen occurs in a nascent state and is reactive and could therefore result in toxic substances with a possible contamination of the water. The daily dose of Hydrogen enriched water was between 250 ml and 1.000 ml.

After starting treatment with Hydrogen enriched water the CFS symptoms gradually improved and after four weeks the symptoms were completely abrogated. Muscle strength and motor function improved substantially and physical strength returned completely. The patient regained the ability to sleep through the night. The patient now scored negative on all diagnostic CDC criteria for CFS (Fukuda *et al.* 1994), i.e. the chronic fatigue had disappeared, as were substantial impairment in short-term memory and concentration, sore throat, headache, unrefreshing sleep, post exertion malaise and muscle pain. This means that he no longer suffered from CFS as diagnosed with the CDC criteria. An antihypertensive treatment with Ramipril 5 mg twice a day restored the status of the patient before the onset of LICI.

Although XX no longer suffered from chronic respiratory symptoms and CFS, after remaining in a hardware store for one hour in April 2013 his vocal chords and larynx reacted and a mild fatigue during the three consecutive days re-occurred. Therefore, a maintaining therapy with Hydrogen enriched water is needed to minimize the risk of recurrent short episodes of hypersensitivity and fatigue reactions. July 2013 XX is again professionally active and can resume his activities as a scientific researcher.

All in all, this patient developed a combination of airway hyperesponsiveness and CFS following exposure to LP&P. The symptom cluster he developed is in accordance with the symptoms described by the International Foundation "nano-Control", Hamburg, Germany, which considers airway hypersensitivity and CF/CFS as key characteristics of this condition. His symptoms were aggravated by acute exposure to LP&P. Running nose, chronic cough, burning tongue and swollen mucosa of the nose and tongue were other signs of the hyperresponsivity / hypersensitivity reactions or irritation, whereas headache, word finding difficulties, sadness, massive fatigue, muscle weakness and

aches, and sleep disorders are characteristics of CFS. It is interesting to note that the symptoms emerged after changing all LP&P devices in the office room where XX was working. It is indeed known that LP&P emissions in an indoor environment may increase submicrometer particle concentrations in the office room in a printer-specific manner, i.e. depending on the printer a very low to high LP&P emission may be recorded (He *et al.* 2007; Betha *et al.* 2011).

XX not only showed characteristic symptoms belonging to the two major symptoms clusters, i.e. CFS and lower and upper respiratory tract symptoms, but also hypertension, reactions to metals and alopecia areata. As described above, inflammatory and O&NS pathways play a major role in hypertension (Ghanem and Movahed, 2007). Interestingly, there is a strong comorbidity between CFS and cardiovascular disorder (Maes and Twisk, 2009). The metals in LP&P emissions may, by causing inflammation, O&NS and modulating the TLR4 complex, contribute to the clinical picture. Pro-inflammatory cytokines, such as TNFα and IFNγ, and O&NS processes, including lipid peroxidation and defective superoxide dismutase activity, play an important role in the pathophysiology of alopecia areata (Gregoriou *et al.* 2010; Abdel-Fattah *et al.* 2011). A significant genome-wide association between innate immune system (autoimmunity, inflammatory markers) and alopecia areata is described (Petukhova *et al.* 2010).

Abstention from LP&P emissions for more than one year only slightly improved the hypersensitivity reactions and had no effect on CFS, indicating that a chronic condition had developed with acute exacerbations following LP&P exposure. Treatments, however, with Cinnamon and Hydrogen significantly improved LP&P-induced symptoms. Thus, treatment with Cinnamon extract abrogated the hyperresponsiveness of the upper and lower respiratory tract, but not CFS, while treatment with Hydrogen improved the CFS. These effects may be explained since Cinnamon extract is a MyD88 antagonist, while Hydrogen is a potent antioxidant and antiinflammatory agent. Thus, both treatments may attenuate the TLR4 Radical Cycle, which may underpin the pathophysiology of LICI, and thus the accompanying symptoms, including airway hyperresponsiveness, CFS, hypertension and alopecia areata.

7. DISCUSSION

Exposure to LP&P to vulnerable patients may cause a symptom complex consisting of two major symptomatic factors, i.e. hyperresponsiveness and irritation of the upper and lower respiratory tract and chronic fatigue (syndrome). The emissions of LP&P are complex. While many substances can be found in the toner other chemicals and nanoparticles are generated during the printing process. One major problem is that there is virtually no access to the exact composition of toners and the additives used. In fact, hundreds of compositions can be found in patented publications. Substances that can be found in most toners or during the printing process are Silica nanoparticles, Titanium Dioxide nanoparticles, Carbon black, metals (e.g. Magnetite or iron (II,III) oxide, Fe2+Fe3+2O4, titanium, chromium, nickel, aluminum, cobalt, ozone, volatile organic compounds (VOC), etc. We have reviewed that most of these substances generate free radicals, oxidative stress, inflammatory reactions, DNA repair deficiencies, or directly stimulate cells through TRL4-related mechanisms. Therefore LP&P emissions may cause activation of the TLR4 Radical Cycle and thus be associated with onset of chronic inflammatory and oxidative disorders, such as CFS. This also explains the clinical efficacy of Cinnamon and Hydrogen in this condition. Thus, Cinnamon is an antagonist of MyD88, an intracellular messenger molecule of the TLR2 and TLR4 complex. Hydrogen is a strong antioxidant and antiinflammatory agent that has a high bioavailability. High doses of Cinnamon extract and Hydrogen may attenuate TLR4 complex signaling and therefore improve chronic inflammatory and O&NS conditions, such as LP&P-induced CFS and airway hyperresponsiveness.

Conflict of interest

Kurt Lucas has filed two relevant patent applications, i.e. (October 2011) Cinnamon Extract for the treatment of diseases caused by induced mismanagement of the innate immune system; and (September 2012) Compositions for the preparation of hydrogen enriched water.

MM does not report any conflict of interest.

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