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ORIGINAL ARTICLE

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Biomarkers and chemosensory irritations

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Abstract ***Objectives***: A literature review on studies in humans, applying physiological methods to monitor environmentally induced reactions in eyes and upper respiratory tract. The focus was on chemical exposures, but other occupational factors and indoor exposures were included. ***Methods***: Original articles were gathered from Medline until November 2000, combined with peer-reviewed publications from other sources. ***Results***: Ocular methods included measurement of tear film break-up time (BUT), blink frequency, detection of corneal damage, by vital staining, and cells or inflammatory markers in tear fluid. Nasal methods included acoustic rhinometry, rhinostereometry, and nasal peak expiratory flow. In addition, nasal lavage with isotonic sodium chloride solution was applied to measure concentrations of leucocytes, or biomarkers of secretion or inflammation in nasal lavage fluid (NAL). Most occupational studies were on nasal effects of organic or inorganic dust. There were few studies on occupational exposure to organic solvents or chemical irritants. Some studies demonstrated associations between ocular and nasal physiological response and the indoor environment. Finally, there were some exposure-chamber studies on effects of specific volatile organic compounds (VOCs). Little is known about adaptation at repeated ocular or nasal exposure to irritants. ***Conclusion***: Physiological measurements can be valuable complements to symptom registration, but there is a need for standardised investigations. There is a lack of studies on ocular and nasal physiological responses in relation to specific chemical compounds. Experimental studies, with repeated exposure and longer follow-up time on biomarkers, are needed. Finally, there is a need for longitudinal epidemiological studies to elucidate if observed effects should be interpreted as variation within normal physiology, or as early signs of impaired ocular and respiratory health.

Keywords Biomarkers · Dust · Indoor environment · Inflammation · Nasal patency · Sensory irritation · Volatile organic compounds · Occupational exposure · Organic solvents · Ocular effects

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Introduction

Many current occupational limit values for chemical compounds are based on sensory irritation. The perception of airborne chemical substances relies on two sensory channels: olfaction and the common chemical sense. The olfactory nerve (cranial nerve I) subserves the perception of odour, and the trigeminal nerve (cranial nerve V) subserves the perception of pungency and common chemical sensations (Commeto-Muñiz and Cain 1991). Alarie (1966) proposed that the trigeminal nerve endings in the nasal mucosa of mice could be used in an experimental model to study irritating properties of airborne chemical pollutants. He found a good correlation ($R^2 = 0.89$) between logarithmic values for existing threshold limit values (TLVs) in the USA, and trigeminal irritation in the mice model (Alarie 1981). Other researchers have performed exposure-chamber studies to study olfactory adaptation (Dalton 2000), or compared subjects with normal olfaction (normosmics) with those lacking it (anosmics) (Commeto-Muñiz and Cain 1991). Another approach is to combine symptom reporting with toxicokinetic studies in exposure-chamber studies (Åkesson and Paulsson 1997; Falk et al. 1991; Järnberg et al. 1996). The clinical significance of sensory hyperreactivity has been demonstrated in non-asthmatic patients with respiratory symptoms. By capsaicin provocation in the upper respiratory tract, individuals with increased reactivity to trigeminal

irritants were identified (Millqvist 2000). They reacted with asthma-like symptoms and cough on exposure to low levels of irritants (Millqvist et al, 1999).

Symptom reporting can be influenced by factors other than the exposure levels, including attitudes to the exposure. The significance of cognitive bias was demonstrated in an exposure-chamber study. Three comparable groups were exposed to the same chemical in the same concentration, but were given different information. Those informed that they were exposed to an industrial chemical exhibited more symptoms than those who received neutral information, or were informed that they were exposed to a natural product (Dalton et al., 1997). One alternative to symptom reporting is to study physiological reactions as endpoints, in the work to establish occupational exposure limit values for chemical irritants. This publication gives a short overview of methods that are available to study physiological responses in the eyes and upper respiratory tract, in relation to environmental exposure. Secondly, it gives a review of publications applying these methods in epidemiological or human experimental studies on environmental exposure in the workplace in a broad sense, and in particular, exposure to chemical irritants. Finally, strengths and weaknesses of these physiological methods will be discussed, as well as knowledge (or lack of knowledge) of effect-response relationships, reversibility, adaptation, habituation, and influence of individual differences.

Ocular physiological methods

Methods available to study ocular physiological reactions to environmental exposure have been reviewed by Norn (1992) and Kjaergaard (1992). Provocation with carbon dioxide (CO_2) has been used to measure trigeminal sensitivity in the eyes (Kjaergaard et al. 1992). Other methods include photographic measurement of eye redness or blink frequency, foam formation in inner canthus, or tear production. Tear film stability can be studied, by measuring the tear film break-up time (BUT), either by fluorescin staining and ocular microscopy, or by recording the time the subject can keep the eyes open without pain, when watching a fixed point at the wall (self-reported BUT). Self-reported BUT has a good correlation with the fluorescein method (Wyon and Wyon 1987). Conjunctival or corneal epithelial damage can be measured by vital staining with 1% rose Bengal or Lissamine green. Finally, conjunctival cytology can be applied, and biomarkers can be measured in tear fluid.

Nasal physiological methods

Inflammation of the nasal mucosa evokes nasal obstruction, which is a *symptom* that can be perceived for different reasons (Jessen and Malm 1997). Nasal patency denotes an *objective measure* of how open the nose is. Methods to measure nasal patency include: computed tomography, magnetic resonance imaging, volumetry, rhinostereometry, and acoustic rhinometry (Malm 1997). Acoustic rhinometry is a convenient method to use in environmental studies and can be combined with the spraying of a nasal decongestive adrenergic substance (xylometazoline-hydrochloride) to measure reversible nasal decongestion (Wålinder et al. 1997). Triplicate measurements have shown a relative standard error of variation of 6%–9% for acoustic rhinometry (Wålinder et al. 2000). Rhinostereometry is another method used to measure nasal patency. It can be combined with histamine challenge to measure non-allergic nasal hyperreactivity (Hallen and Juto 1994). Other functional measures include rhinomanometry and nasal peak flow measurements (Malm 1997).

Nasal lavage with isotonic sodium chloride (NaCl) solution has been used to study inflammatory effects in the nasal mucosa at inhalatory exposure (Koren et al., 1990; Wilcosky 1993). Other methods used to sample nasal secretion include nasal spray-washing, or absorption on cotton wool or rubber foam samplers (Klimek and Rasp 1999). Initially, cytological investigations of nasal lavage fluid (NAL) was common (Koren and Devlin 1992; Hauser et al. 1994). Hauser et al. (1994) reported that within-subject variability is smaller than between-subject variability in NAL investigations. Later, biomarkers of allergy or inflammation have been measured in NAL. There is a large number of possible biomarkers, including tryptase, albumin, lysozyme, eosinophilic cationic protein (ECP), and myeloperoxidase (MPO). Tryptase is a marker of mast-cell activation in type-1 allergy (Castells and Schwartz 1988). Albumin in NAL can be an indicator of vasomotor response of the nasal mucosa, with plasma exudation from endothelial gaps in post-capillary venules (Wålinder et al. 2000). Lysozyme is secreted from several sources: nasal submucosal glands, activated macrophages, and neutrophil granulocytes (Raphael et al. 1989). In cytological analysis of nasal lavage, approximately 90% of leucocytes are neutrophilic granulocytes (Pipkorn et al. 1989), and MPO has been shown to be a specific biomarker for the activity of neutrophilic granulocytes (Schmenkel et al. 1990). The level of ECP in NAL is a measure of the activity of eosinophilic granulocytes. This granular protein is cytotoxic and can be destructive to the respiratory epithelium (Venge et al. 1989). The methodological variation, measured as day-to-day variability (CV) for biomarkers in NAL has been reported to be 69% for ECP, 42% for lysozyme, 50% for MPO, and 83% for albumin (Wålinder et al. 2000). Other biomarkers that have been monitored in NAL include different cytokines/interleukins, including TNF-alpha, IL-1beta, IL-6, IL-8, and prostaglandin E(2) (Muttray et al. 1999; Hirvonen et al. 1999), and neuropeptides such as substance P (Schultz et al. 1996). The concentrations of nitrite or nitrate in NAL can be used as biomarkers of nitric oxide (NO) formation in the nasal cavity (Hirvonen et al. 1999) Finally, environmental influence

on antioxidants in the nasal mucosa can be monitored by measurement of antioxidants in NAL, e.g. ascorbic acid, uric acid, glutathione (GSH), and alpha-tocopherol (van der Vliet et al. 1999). Other methods used to study nasal mucosal effects include measurement of mucocililar clearance, e.g. by the saccharin test (Toren et al. 1996), or ciliary beat frequency with video-interference contrast microscopy (Muttray et al. 1999).

Ocular physiological effects of specific chemical compounds

There are few publications measuring ocular physiological effects of experimental exposure to single volatile organic compounds. In one early work by Kjaergaard et al. (1989), healthy subject were exposed to *n*-decane at 0, 10, 35, and 100 ppm for 6 h in a controlled, double-blind study using a Latin-square exposure design. Using the fluorescein staining method, they found decreased tear film BUT at all exposure levels. Moreover, the number of conjunctival polymorphonuclear leucocytes increased in conjunctival secretions in a dose-related way. In another experimental study, male subjects were exposed to ethyl-*tert*-butyl ether (ETBE) vapour for 2 h at four exposure levels (0, 5, 25 and 50 ppm) during physical exercise. Increased blink frequency was observed at all exposure levels, with the magnitude of change related to the exposure level (Nihlén et al. 1998a). A similar study was performed with methyl-*tert*-butyl ether (MTBE) vapour for 2 h at three levels (5, 25, 50 ppm). No ocular effects of MTBE could be detected. (Nihlén et al. 1998b). In another experimental study, subjects were exposed to either 1-octene at four levels (0, 99, 314, 990 ppm), or *n*-butanol at four levels (0, 1,307, 2,266, 3,922 ppm). Perceived intensity of eye irritation and conjunctival hyperaemia increased with increased exposure levels, whereas cytological changes in the conjunctival fluid were not related to the exposure (Hempel-Jørgensen et al. 1998). Finally, healthy adult non-smokers were exposed to nitrous acid (HNO_2) for 3.5 h at three exposure levels (0, 77, 395 ppb). They reported an exposure-related change in tear-fluid cytology (Rasmussen et al. 1995).

Ocular physiological effects in relation to workplace factors

In a study on workers in the Danish tobacco industry, with matched office workers as controls, a dose-dependent effect on photographically measured eye redness was observed. Moreover, tobacco workers had a higher concentration of most cell types in eye cytology than did controls (Kjaergaard and Pedersen 1989). Environmental tobacco smoke (ETS) may also influence the ocular mucosa. Decreased tear film stability has been observed in airline crew exposed to ETS in aircraft cabins (Wieslander et al. 2000). Decreased tear film stability has been observed in house painters exposed to water-based paints, compared with unexposed janitors (Wieslander and Norbäck, 1998).

Objective eye manifestations have been reported to be more common in office workers than in industrial workers or the general population, suggesting specific ocular effects from the office environment (Franck et al., 1993). Video display work has been shown to decrease blink frequency and increase the occurrence of ocular symptoms (Acosta et al. 1999), and exposure to emissions from office machines has been shown experimentally to increase epithelial damage of the conjunctiva (Wolkoff et al. 1992). Experimental exposure to a standard mixture of 22 different volatile organic compounds normally found in Danish houses has been shown to increase the concentration of albumin in tear fluid (Thygesen et al. 1987). There are also indications that building-related exposures may influence ocular physiological signs. Muzi et al. (1998) found that office workers in a modern air-conditioned building had reduced tear film stability, compared with office workers in an older naturally ventilated building. In a study on signs of eye irritation in female hospital workers, decreased tear film stability (BUT) was related to alteration of the microbial flora in the indoor air, presence of *Aspergillus fumigatus*, increased dust settlement rate, and urban vicinity of the workplace (Smedbold et al. 2001a). Decreased tear film stability has been reported in office workers in two hospital buildings with dampness in the floor construction, causing emission of 2-ethyl 1-hexanol into the indoor air, due to degradation of the plasticiser di-ethylhexyl phthalate (DEHP) in the floor material (Wieslander et al. 1999b).

Nasal physiological effects of specific chemical compounds

Experimental exposure to 400 ppb of ozone for 2 h was reported to have increased the concentration of tryptase and albumin in NAL (Graham and Koren 1990). In another study, 13 volunteers were exposed to ozone (0, 0.2 ppb) for 2 h; depletion of uric acid in NAL was found after a 2-h exposure to 200 ppb of ozone (Mudway et al. 1999). An increase in albumin and total protein in NAL and the proportion of eosinophils in NAL were reported after a 2-h experimental exposure to 0.5 mg/m^3 of formaldehyde (Pazdrak et al. 1993). Muttray et al. (1999) exposed healthy, non-smoking students for 4 h to 1,1,1-trichloroethane at two levels (20, 200 ppm). Concentrations of IL-1beta, IL-6, and IL-8 in nasal secretion increased on exposure to 200 ppm. In the study of ETBE exposure (0, 5, 25, 50 ppm) mentioned above, decreased nasal patency could be detected at all exposure levels, with no relationship to the exposure levels (Nihlén et al. 1998a). In the MTBE study, nasal airway resistance measured by peak expiratory flows increased after exposure, but no dose-effect relationship was found (Nihlén et al. 1998b).

Nasal physiological effects in relation to workplace factors

Nasal physiological effects at occupational exposure have been studied mainly in relation to particulate pollutants. Nasal lavage was performed before and after workplace exposure to fuel-oil ash containing vanadium pentoxide in workers (boilermakers), with utility workers as unexposed controls. A significant increase in polymorphonuclear cells in NAL was found in non-smoking workers, compared with non-smoking controls. The exposure levels were 0.05–4.51 mg/m^3 for PM_{10}, and 0.0001–0.139 mg/m^3 of vanadium (Hauser et al. 1995). In a later study on vanadium-exposed workers, biomarkers in NAL were compared between exposed boilermakers and non-exposed controls: MPO and IL-8 in NAL were increased on exposure. The changes were associated with PM_{10} levels in air (0.10–0.47 mg/m^3), and concentrations of vanadium in NAL (Woodin et al. 1998).

In one longitudinal study, nasal signs were studied in pulp-mill workers exposed to lime dust (calcium oxide), and matched unexposed referents. The study was repeated 1 year later, after work-environment improvements. The mean total dust level was 1.2 mg/m^3 initially, and was reduced to 0.1 mg/m^3 after the improvements. The saccharin test showed impaired mucociliary function in exposed workers initially, and when dust levels were reduced, mucociliary function was improved (Toren et al. 1996). Effects of sawmill air contaminants were investigated in naïve healthy non-smokers with no previous employment in wood processing. The participants were investigated before and after 5-h exposure in the sawmill. Ten subjects used respirators with particle filters (total dust: 0.04 mg/m^3; terpene exposure: 58 mg/m^3), nine used respirators without particle filters (total dust: 0.13 mg/m^3; terpene exposure: 52 mg/m^3). The concentration of IL-6 in NAL increased during the working day in those not wearing a particle filter, but was unchanged in those wearing particle filters. Moreover, the change was related to measured dust concentration (Dahlqvist et al. 1996). Another study on exposure to wood dust was performed on industrial-art teachers, using other teachers from the same schools as control subjects. The art teachers had more nasal symptoms but had similar concentrations of biomarkers in NAL. Within the art-teacher group, a correlation was found between the percentage of neutrophils in NAL and the number of classes per week (Åhman et al. 1995). In another study on industrial-art teachers, nasal expiratory flow showed a higher level of nasal blockage in woodwork teachers than in controls, with gradually decreased nasal peak flow during the working week (Åhman and Söderman 1996). Paper-mill workers exposed to soft paper dust (mean: 3.9 mg/m^3 of dust) had more nasal symptoms than unexposed controls, but there was no evidence of decreased nasal patency or reduced mucociliary clearance in exposed workers (Hellgren et al. 2001).

Other studies have shown nasal effects of occupational exposure to different types of organic dust. In bakers exposed to 1.0–3.8 mg/m^3 of inhalable dust, there were no apparent differences in NAL biomarkers between bakers and controls, but there was a positive correlation between cumulative dose of inhalable flour dust and NAL levels of MPO and hyaluronic acid (Brisman et al. 1998). Healthy non-smokers not previously exposed to swine dust were exposed to normal farm work for 3 h in a piggery. They had strong nasal reactions, with a 19-fold increase in neutrophil concentration, and a sevenfold increase in IL-8 in NAL, and decreased nasal patency. The air concentration of inhalable dust was 20–29 mg/m^3, endotoxin 1.1–1.4 μg/m^3, and muramic acid 0.78–2.1 μg/m^3 (Larsson et al. 1997). In a later similar study, naïve subjects were exposed for 3 h during work in poultry houses, with 2–4 mg/m^3 of inhalable dust and mean concentrations of 10 ng/m^3 of endotoxin. A significant increase in IL-6 in NAL was observed (Larsson et al. 1999). In compost workers, total cells, MPO, IL-8, NO, and albumin were increased in NAL during the workshift in exposed workers, compared with workshift changes in unexposed controls. Mean total dust exposure was 0.4–3.3 mg/m^3, and endotoxin levels were 50–1,000 endotoxin units (EU)/m^3 (Douwes et al. 1997).

We found only a few studies on nasal effects of chemical exposure in the workplace. In one study, where workers were exposed to methyl tetrahydrophthalic anhydride (MTHPA), workers sensitised to MTHPA had significant levels of tryptase in NAL but no increase in ECP (Nielsen et al. 1994). In another study, workers in the wood-surface coating industry exposed to UV-curing acrylate coatings had increased levels of ECP in NAL, compared with unexposed controls. In addition, there was a positive correlation between exposure time and ECP and albumin levels in NAL (Granstrand et al. 1998). In the previously mentioned study of house painters exposed to emissions from water-based paints, non-asthmatic house painters had increased lysozyme in NAL, compared with non-asthmatic controls. In addition, there was correlation between ECP in NAL and the number of hours/week exposure to water-based paints (Wieslander and Norbäck 1998).

There are also recent studies on nasal effects of the indoor environment. Decreased nasal patency and increases in lysozyme and ECP in NAL were observed in individuals in schools with room temperatures above 22 °C (Wålinder et al. 1998), and with low air exchange rates (Wålinder et al. 1998), and low cleaning frequency (Wålinder et al. 1999). In addition, decreased nasal patency was associated with higher levels of formaldehyde, nitrogen dioxide, and respirable dust in the classrooms (Norbäck et al. 2000) Moreover, ECP and lysozyme was higher at higher indoor concentrations of formaldehyde and nitrogen dioxide (Norbäck et al. 2000). Finally, in a longitudinal study, migration to a new office building, recently painted with a new type of water-based paints, was related to an increase in ECP and lysozyme in NAL,

and increased nasal patency (Wieslander et al. 1999a). Some studies indicate that microbial or chemical exposure related to building dampness could influence the nasal mucosa. Increases in ECP, MPO and albumin in NAL were observed in a building with pronounced microbial growth in the structure (including *Stachybotrys spp.*) (Wålinder et al. 2001). A lower degree of nasal patency and increases in ECP and lysozyme in NAL were found at higher concentrations of total moulds and in the presence of *Aspergillus spp.* in classroom air (Norbäck et al. 2000). An association between presence of *A. fumigatus* in indoor air, and decreased nasal patency was reported in hospital workers (Smedbold et al. 2001b). In two geriatric hospitals, dampness in the floor construction and presence of 2-ethyl-1-hexanol in indoor air was associated with an increase of lysozyme in NAL (Wieslander et al. 1999b). Finally, teachers in Finland working in a mouldy school building had higher concentrations of TNF-alpha, IL-6 and NO in NAL than did unexposed controls, and the biomarker concentrations were normalised during summer vacation (Hirvonen et al. 1999).

Influence of personal factors

Individual sensitivity to irritants can be verified by different tests. The stinger test is used to screen for subjects prone to get skin irritation (Kjaergaard et al. 1989); the carbon dioxide test has been used to screen for ocular trigeminal sensitivity (Kjaergaard et al. 1992); and the capsaicin test for respiratory trigeminal sensitivity (Millqvist et al. 1999). Little is known of the relation between individual sensitivity to irritants, as measured by these tests, and physiological response measured as biomarker concentrations in nasal lavage, or degree of nasal patency. There is some information on individual variation of nasal physiological signs. In the soft paper dust study, IL-8 in NAL was 46% higher in men than in women, and twice as high in smokers than in non-smokers (Hellgren et al. 2001). In white-collar workers, men had 10% higher concentration of ECP, 200% higher concentration of MPO, and 60% higher concentration of albumin in NAL In contrast, women had 8% smaller nasal volume and 12% smaller minimum cross-sectional area in the anterior part of the nasal cavity. Rhinometric dimensions or lavage fluid biomarker concentrations were not related to age, smoking, asthma, or a history of atopy (Wålinder al. 2000).

Relationships between symptoms and physiological signs

A relationship between ocular symptoms and signs has been reported in different studies. Franck (1986) found that office workers reporting ocular symptoms had decreased tear-film stability as measured by the fluorescein method, and signs of epithelial damage indicated by ocular microscopy after staining with Lissamine green. Hospital workers with ocular symptoms, both during the past week and the past 3 months had decreased tear film stability, measured as self-reported BUT. In this study, there was also a positive relationship between nasal symptoms in the past week and MPO in NAL (Wieslander et al. 1999b). Åhman et al. (1995) found higher concentrations of albumin in NAL in wood art teachers complaining of nasal stuffiness, and bakers with runny noses had higher concentrations of MPO and hyaluronic acid in NAL (Brisman et al., 1998). Workers in a wood-surface coating industry reporting nasal symptoms had increased concentration of ECP in NAL, compared with non-symptomatic workers (Granstrand et al. 1998). In a study on 18 patients with perennial rhinitis, a correlation was found between nasal symptom score and nasal mucosal swelling measured by rhinostereometry using a histamine challenge test (Hallen and Juto 1994). Finally, in the study on white-collar workers, the anterior nasal volume was decreased among those reporting nasal obstruction (Wålinder et al. 2000).

Conclusion

During the past decade, several epidemiological studies have been published assessing relationships between environmental exposure and physiological signs from the eyes and nasal mucosa. Most of these studies are small, typically with 10–40 exposed subjects and a similar number of controls, with limited statistical power due to inter- and intra-individual variation of the physiological measurements. Other limitations of most studies are that they are either small cross-sectional epidemiological studies, or experimental studies with a single exposure for a few hours and a follow-up time of a few hours. Despite these limitations, significant effects of environmental exposure have been demonstrated, and in some cases even a dose-effect relationship. Most occupational studies have been dealing with nasal effects of organic or inorganic dust, and few studies are available on occupational exposure to organic solvents or other chemical irritants. Limited data suggest that occupational exposure to volatile organic compounds could have physiologically measurable effects on ocular and nasal mucosa. Studies from non-industrial workplaces (e.g. schools, offices, hospitals) have demonstrated associations between different indoor exposures and factors, and physiologically measurable effects on the eye and upper respiratory tract. Very little is known about adaptation at repeated exposure, with regard to physiological reactions in the eyes or the upper respiratory mucosa. In conclusion, biomarkers and other physiological measures can be valuable complements to symptom registration when occupational exposure limit values have to be set. There is, however, an obvious lack of data on ocular and nasal physiological effects of exposure to volatile organic compounds, organic solvents, or other chemical irritants. More experimental exposure-chamber studies are needed, with longer follow-up times and repeated exposure. There is also a

need for standardisation of physiological methods and population characteristics of participants in experimental studies, with respect to gender, age, smoking habits, allergy, and respiratory disorders, in order to make different studies comparable. Finally, longitudinal epidemiological studies in workplaces are needed, to elucidate if the observed effects should be interpreted as variation within normal physiology, or early signs of impaired ocular and respiratory health.

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