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## Review:

# Nasal Toxicity, Carcinogenicity, and Olfactory Uptake of Metals

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**Abstract.** Occupational exposures to inhalation of certain metal dusts or aerosols can cause loss of olfactory acuity, atrophy of the nasal mucosa, mucosal ulcers, perforated nasal septum, or sinonasal cancer. Anosmia and hyposmia have been observed in workers exposed to Ni- or Cd-containing dusts in alkaline battery factories, nickel refineries, and cadmium industries. Ulcers of the nasal mucosa and perforated nasal septum have been reported in workers exposed to Cr(VI) in chromate production and chrome plating, or to As(III) in arsenic smelters. Atrophy of the olfactory epithelium has been observed in rodents following inhalation of NiSO<sub>4</sub> or αNi<sub>3</sub>S<sub>2</sub>. Cancers of the nose and nasal sinuses have been reported in workers exposed to Ni compounds in nickel refining, cutlery factories, and alkaline battery manufacture, or to Cr(VI) in chromate production and chrome plating. In animals, several metals (eg, Al, Cd, Co, Hg, Mn, Ni, Zn) have been shown to pass via olfactory receptor neurons from the nasal lumen through the cribriform plate to the olfactory bulb. Some metals (eg, Mn, Ni, Zn) can cross synapses in the olfactory bulb and migrate via secondary olfactory neurons to distant nuclei of the brain. After nasal instillation of a metal-containing solution, transport of the metal via olfactory axons can occur rapidly, within hours or a few days (eg, Mn), or slowly over days or weeks (eg, Ni). The olfactory bulb tends to accumulate certain metals (eg, Al, Bi, Cu, Mn, Zn) with greater avidity than other regions of the brain. The molecular mechanisms responsible for metal translocation in olfactory neurons and deposition in the olfactory bulb are unclear, but complexation by metal-binding molecules such as carnosine (β-alanyl-L-histidine) may be involved. (received 2 October 2000; accepted 16 November 2000)

**Keywords:** Rhinotoxicity, anosmia, nasal perforation, sinonasal cancer, olfactory nerve, olfactory bulb, aluminum, arsenic, bismuth, cadmium, chromium, cobalt, copper, mercury, manganese, nickel, zinc

## Introduction

Monographs on metal toxicology point to the respiratory system as one of the primary targets of metal toxicity, but they tend to focus on the lung and neglect the upper airways [1-5]. This article reviews the rhinotoxicity of metals in order to alert physicians and toxicologists to the importance of the nose, paranasal sinuses, and olfactory system as targets of metal toxicity. In addition to their role in olfaction, the nasal passages provide some protection to the lower respiratory tract by filtering the inspired air. This

filtering action, however, places nasal structures at risk for toxic injury and neoplasia [6,7].

This article begins by reviewing three clinical topics: (a) anosmia and hyposmia in workers who have chronically inhaled cadmium or nickel compounds; (b) nasal mucosal ulceration and perforation of the nasal septum in workers who have chronically inhaled hexavalent chromium or trivalent arsenic compounds; and (c) sinonasal cancer in workers who have chronically inhaled certain nickel or chromium compounds. The second half of the article reviews animal investigations, focusing specifically on (d) nasal mucosal atrophy induced by inhalation of nickel compounds; (e) passage of metals from the nasal lumen

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to the brain via olfactory receptor neurons; (f) the possible role of carnosine ( $\beta$ -alanyl-L-histidine) as a metal-binding constituent of olfactory cells; and (g) the propensity of the olfactory bulb to accumulate metals, in comparison to other regions of the brain. The article concludes with some recommendations for practitioners of occupational and environmental medicine, and a discussion of current gaps in knowledge and prospects for future research.

Inspired air enters the nose through each nostril and flows in a laminar fashion over the turbinate bones, through the nasopharynx, and into the trachea. A minor fraction of the inspired air flows into the nasal vault and passes over the olfactory neuroepithelium, which is situated in a cleft on the undersurface of the ethmoid bone [8,9]. By convection and diffusion, some inspired air also enters the ostia of the nasal sinuses. The olfactory neuroepithelium, approximately 3 cm<sup>2</sup> in area, contains several cell types, including (a) olfactory receptor neurons, (b) sustentacular cells, (c) mucus cells, (d) microvillar cells, and (e) basal cells [10,11]. Olfactory receptor neurons are bipolar cells; one pole is a dendritic knob that contacts the nasal mucus and absorbs odorants by endocytosis.

The transduction cascade of odorant detection is initiated by chemoreceptors on cilia of the dendritic knob [12]. Coding regions for 330 odorant chemoreceptor genes, including pseudogenes, have been identified in the human genome database. The subgenome that encodes odorant chemoreceptors comprises the largest known gene family in vertebrates, providing recognition capacity for millions of potential odorants [13].

The other pole of olfactory receptor neurons projects a long axon that penetrates the basement membrane, traverses the cribriform plate of the ethmoid bone, enters the cranial cavity, and forms synapses with secondary olfactory neurons in glomeruli of the ipsilateral olfactory bulb [10-12,14]. Axons of secondary olfactory neurons project to the thalamus, hypothalamus, hippocampus, olfactory tubercle, pyriform, amygdala, and other areas of the brain [10,11,15].

Olfactory receptor neurons are unique among neural cells in two important respects: (a) they contact directly both the external environment and the brain [16], and (b) they regenerate from basal cells following

damage [17]. That neurotropic viruses can pass from the nose to the brain along the olfactory pathway has been postulated since the 1930s [18,19]. The so-called "nose-brain barrier," which impedes the translocation of viruses and toxicants, comprises several components, including the physical obstacle of nasal mucus, the mobilization of nasal secretions by the mucociliary apparatus, the immunological defenses of the nasal mucosa, the tight junctions between olfactory receptor neurons and sustentacular cells, the xenobiotic metabolizing activity of sustentacular cells, and the cellular desquamation that follows toxicant exposure [10,16].

### Olfactory Deficits in Metal-Exposed Workers

Olfactory disorders include complete loss of smell sensation (anosmia), partial loss of smell sensation (hyposmia), perverted sense of smell (dysosmia), and perception of phantom odors (phantosmia). Clinical reports of impaired olfaction in metal-exposed workers are compiled in Table 1. In 1948, Friberg [20] reported that 37% of workers at an alkaline battery plant in Sweden complained of anosmia. This observation was corroborated by studies in Germany [22], the United Kingdom [23,24], and Poland [25,26] that documented olfactory deficits in 27% to 65% of workers with heavy inhalation exposures to cadmium oxide and nickel hydroxide, the principal chemical constituents of alkaline batteries. At the autopsy of one such worker, Baader [22] observed bright yellow staining of the olfactory bulbs, which suggested that cadmium entered the brain via the olfactory pathway. Most authors attributed the anosmia and hyposmia of battery workers to cadmium toxicity, but Adams and Crabtree [23] concluded that the working conditions in alkaline battery factories make it impossible to determine whether olfaction is impaired by exposure to powdered cadmium oxide, nickel hydroxide, or both.

Olfactory deficits have also been noted in workers who were heavily exposed to compounds of nickel or cadmium in other industrial operations, including electrolytic nickel refineries in the Soviet Union [27,28], a cadmium smelter in China [29], and a refrigerator coil factory in the USA [30]. Recently, Suruda [31] reported anosmia in two Cd-exposed workers who were diagnosed in an office practice of

Table 1. Olfactory impairment in workers exposed to nickel and/or cadmium.

Metal	Country	Exposures & subjects	Observations	Authors & yr
Ni, Cd	Sweden	Ni/Cd battery factory; 43 men with mean exposure of 20 yr	16/43 workers (37%) complained of impaired sense of smell; nasal mucosal atrophy was noted in 10 workers.	Friberg [20,21] (1948, 1950)
Ni, Cd	Germany	Ni/Cd battery factory; man with 16 yr exposure, and anosmia for 10 yr	Autopsy revealed markedly atrophic nasal mucosa and bright yellow staining of olfactory bulbs.	Baader [22] (1952)
Ni, Cd	United Kingdom	Ni/Cd battery factory; 106 workmen employed for a few wk to > 30 yr	Phenol detection test identified severe hyposmia in 27% of exposed workers, vs 5% of control workers.	Adams & Crabtree [23] (1961)
Ni, Cd	United Kingdom	Ni/Cd battery factory; 70 workmen with exposures from 10 to 40 yr	Hyposmia in 64% of all workmen and in 10 of 11 workmen (91%) with exposures from 30 to 40 yr.	Potts [24] (1965)
Ni, Cd	Poland	Ni/Cd battery factory; 73 workers with 4 to 24 yr exposure (mean = 12.5 yr)	Olfactometry showed hyposmia (26%), parosmia (18%), and anosmia (1%). Blood and urine Cd levels correlated with the olfactory deficits.	Rydzewski et al [25] (1998); Sulkowski et al [26] (2000)
Ni	USSR	Electrolytic Ni refinery; unspecified number of Ni-exposed workers	Frequent olfactory impairment, atrophic nasal mucosa, nasal septal ulceration, and sinusitis.	Tatarskaya [27] (1960)
Ni	USSR	Electrolytic Ni refinery; 458 Ni-exposed workers	Anosmia in 114/251 workers (46%) with chronic sinusitis; less severe loss of smell in other Ni-exposed workers.	Kucharin [28] (1970)
Cd	China	Cd smelter workers; 65 Cd-exposed workers (47 men, 18 women)	11/40 workers (27%) with $\geq 5$ yr exposure to Cd complained of anosmia.	Liu et al [29] (1985)
Cd	USA	Refrigerator coil factory; 55 Cd-exposed workers	Butanol threshold detection test identified 7 men (13%) with moderate to severe hyposmia. Test scores for odorant identification were normal.	Rose et al [30] (1992)
Cd	USA	Patients examined in an office practice of occupational medicine	Routine smell testing identified hyposmia in 2 Cd-exposed workers (auto mechanic; jewelry maker)	Suruda [31] (2000)

occupational medicine by routine testing of olfaction using a panel of microencapsulated odorants.

In clinical studies, cadmium and nickel are the only metals whose compounds have been specifically associated with olfactory impairment. In Japanese patients with Minamata disease, attributed to chronic exposure to methylmercury, the incidence of hyposmia is 30 to 50% [32,33]. However, hyposmia is only one of many manifestations of generalized sensory impairment in methyl mercury poisoning, which include paresthesias and impaired visual, auditory, and taste sensation and sometimes progress to complete blindness and deafness [32].

Two studies suggest that the sense of smell may be augmented after exposure to manganese compounds. Mergler et al [34] reported lower smell thresholds in Mn-exposed workers, compared to controls. Lucchini et al [35] observed that the smell thresholds in Mn-exposed workers decreased with increasing urine Mn levels. These findings may reflect accumulation of Mn in the olfactory bulb, as discussed later in this review.

### Mucosal Ulceration and Perforated Nasal Septum

Perforation of the nasal septum has long been recognized in workers exposed to divalent Hg, hexavalent Cr, or trivalent As compounds. In 1814, powdered mercury fulminate (mercuric dioxycyanide) was introduced in England as a primer in percussion caps and detonators. Workers in explosives factories frequently developed perforation of the nasal septum from chronic exposure to mercury fulminate [36]. By the end of the nineteenth century, the problem of Hg(II)-induced nasal perforation was largely ameliorated by improvements in munitions production [36]. Nasal septum perforations were, however, observed throughout the twentieth century in workers exposed to inhalation of Cr(VI) in chromate production or chrome-plating and those exposed to inhalation of As(III), especially in arsenic and copper smelters (Table 2). These lesions commonly started as an ulceration of the nasal mucosa at a relatively avascular site, 1 cm from the anterior and lower margin of the nasal septum. Typically, the ulceration gradually extended backward and upward; when ulceration occurred on opposite sides of the septum, the cartilage became necrotic and perforation ensued. The

perforated septum was generally painless; the subjects complained of chronic rhinitis and a whistling noise on inspiration, but seldom complained of impaired sense of smell.

In a study of Polish workers with perforation of the nasal septum, the most common causative agents were Cr-containing dust and powdered cement [48]. Occupational exposures to As-, Cd-, Ni-, or fluoride-containing dusts were also implicated in a few cases [48]. At the beginning of the twenty-first century, newly developed ulcers of the nasal mucosa and perforations of the nasal septum have become uncommon, since advances in industrial hygiene have greatly reduced the exposure of workers to inhalation of Cr(VI)- and As(III)-containing dusts. Reports of rhinotoxicity in workers exposed to certain other metals (ruthenium, platinum, copper, and vanadium) are also noted in Table 2.

### Sinonasal Cancer in Ni- and Cr(VI)-Exposed Workers

The propensity of nickel refinery workers to develop sinonasal cancer was reported in 1932 by Bridge [49] and Grenfell and Samuel [50], based on the occurrence of 10 cases at a refinery in Wales. Additional cases were reported by Morgan [51], and an epidemiological study by Doll and coworkers [52] identified 39 deaths from cancers of the nose and paranasal sinuses among workers at the Welsh refinery (Table 3). The present author compiled 139 case reports of sinonasal cancers in nickel refinery workers in Wales, Norway, France, Canada, and the USSR [53]. Meta-analysis of worldwide data by Doll et al [56] attributed the increased risks of sinonasal cancer to inhalation of soluble nickel compounds (eg, NiSO<sub>4</sub>) and certain insoluble nickel compounds (eg, αNi<sub>3</sub>S<sub>2</sub>, NiO, Ni(OH)<sub>2</sub>).

The histological diagnoses of 100 sinonasal neoplasms in nickel refinery workers included squamous cell carcinoma (48%), anaplastic/undifferentiated carcinoma (39%), adenocarcinoma (6%), transitional cell carcinoma (3%), and other malignant tumors (4%) [55]. The nasal neoplasms of nickel refinery workers generally involve the turbinates and the ethmoid or antral sinuses; they are aggressive locally and metastasize widely, so the prognosis is poor [57,58]. The risk of sinonasal cancer from nickel

Table 2. Rhinotoxicity, including mucosal ulceration and septum perforation, in workers exposed to various metals.

Metal	Country	Exposures & subjects	Observations	Authors & yr
Cr(VI)	United Kingdom	176 workers engaged in dichromate production	Perforated nasal septum in 76% and ulcerated mucosa in 11% of workers.	Legge [37] (1902)
Cr(VI)	USA	97 workers engaged in chromate production	Septum perforation in 63%, mucosal ulcer in 6%, nasal polyps in 2%, chronic rhinitis in 87%, sinusitis in 37%, hyposmia in 5%.	Mancuso [38] (1951)
Cr(VI)	United Kingdom	369 chrome platers, exposed for $\geq 5$ yr	Septum perforation in 9%, mucosal ulcer in 17%, recurrent nose bleed in 16%.	Royle [39] (1975)
Cr(VI)	Taiwan	79 chrome platers	Septum perforation in 20%, mucosal ulcer or scars in 53%.	Lin et al [40] (1994)
Cr(VI)	Taiwan	26 chrome platers, mean exposure 6 yr	Septum perforation in 31%, mucosal ulcer in 38%, rhinorrhea in 35%.	Kuo et al [41] (1997)
As(III)	USA	Workers exposed to $As_2O_3$ in a Cu smelter	Clinical findings in 75 workers with nasal mucosal ulcer and septum perforation.	Dunlap [42] (1921)
As(III)	Sweden	1276 workers in an As smelter	Rhinitis and/or septum perforation in 30% of workers exposed to crude or refined As.	Lundgren [43] (1954)
As(III)	USA	Workers exposed to $As_2O_3$ in a Cu smelter	Septum perforation common in workers; approximately 2 new cases diagnosed per year.	Hine et al [44] (1977)
Ru, Pt	United Kingdom	16 women in chemical plant producing Ru & Pt salts	8 women with mucosal ulcer and one with perforated septum after exposure for 2-10 mo.	Harris [45] (1975)
Cu(II)	Sweden	10 sheet metal workers exposed to various Cu salts	All subjects complained of metallic taste and irritated oral or nasal mucosa; atrophy of nasal mucosa was evident in 4 of 10 workers.	Askergrén & Mellgren [46] (1975)
V	Finland	63 workmen in a V smelter, mean exposure 11 yr	Rhinoscopy was normal, but nasal mucosal biopsies showed epithelial hyperplasia and subepithelial mononuclear cell infiltrates.	Kiviluoto et al [47] (1979)
As, Cd Cr, Ni	Poland	185 workers in various industries who developed perforated nasal septum	The septum perforations were attributed to exposures to Cr in 46%, cement in 44%, As in 4%, Cd in 3%, Ni in 1%, and F in 1%.	Kowalska & Sulkowski [48] (1983)

Table 3. Cancers of the nose and nasal sinuses in workers exposed to nickel compounds or chromates.

Metal	Country	Exposures & subjects	Observations	Authors & yr
Ni	Wales	845 Ni refinery workers, employed $\geq$ 5 yr	39 deaths from nasal cancer in workers who were employed before 1925; none in workers who began work from 1925 to 1944.	Doll et al [52] (1970)
Ni	Worldwide	Sinonasal tumors reported in Ni refinery workers	139 cases of sinonasal cancer were compiled from Wales, Norway, France, Canada, & USSR.	Sunderman [53] (1973)
Ni	Canada	54,509 Ni refinery workers	25 deaths from sinonasal cancer (SMRs at two refineries were 3,704 and 7,755, respectively).	Roberts et al [54] (1989)
Ni	Wales, Canada, & Norway	Histopathology records or slides of 100 cancers in Ni refinery workers	Squamous cell carcinoma (48%), anaplastic/undifferentiated ca (39%), adenoca (6%), transitional cell ca (3%), other malignancies (4%).	Sunderman et al [55] (1989)
Ni	Worldwide	Meta-analysis of data for Ni refinery workers	Sinonasal cancer risks were related to sulfidic Ni, soluble Ni, and oxidic Ni, but not metallic Ni.	Doll [56] (1990)
Ni	Sweden	869 workers in Ni/Cd battery factory	3 workers had sinonasal malignancies; the SIR (standardized incidence ratio) was 832.	Jarup et al [59] (1998)
Cr(VI)	United Kingdom	Case report of a chrome pigment worker	An adenocarcinoma of inferior turbinate in association with septal perforation.	Newman [70] (1890)
Cr(VI)	Worldwide	Unspecified number of Cr(VI)-exposed workers	187 deaths from cancer of the respiratory tract included 6 sinonasal cancers.	Hueper [71] (1966)
Cr(VI)	USA	1,200 chromate workers	69 deaths from cancer of the respiratory tract included 2 sinonasal cancers.	Enterline [72] (1974)
Cr(VI)	Japan	896 chromate workers	6 deaths from sinonasal cancer.	Satoh et al [73] (1981)
Cr(VI)	United Kingdom	2,689 workers in Cr/Ni plating operations	3 workers had cancers of the nose or nasal sinuses (the SMR was 1,000).	Sorahan et al [74] (1987)
Cr(VI)	Japan	Case reports of 4 workers in a chromate factory	Description of 4 squamous cell carcinomas, located on the middle turbinate (2), nasal floor (1), or nasopharynx (1).	Satoh et al [75] (1994)

exposure is not restricted to refinery workers; five cases have been reported among workers at alkaline battery and cutlery factories [59-61].

Rhinoscopic examination of nickel refinery workers has revealed varying degrees of hyperplastic rhinitis, especially of the middle turbinates, with polypoid mucosa, distinct polyps, or focal thickening of the mucous membrane, suggestive of neoplasia [62]. Nasal mucosal biopsies of nickel-exposed workers have shown dysplastic and pre-neoplastic lesions (ie, squamous or epidermoid metaplasia), often with immunostaining reactions for keratin and involucrin [63-66]. Torjussen and Andersen [67] found elevated nickel concentrations in nasal biopsy specimens from active and retired nickel workers in Norway. Nickel was retained in the nose for years after cessation of nickel exposure and slowly released; its half-life was 3.5 years. Since there is constant turnover of nasal mucosa cells, the accumulated nickel probably resided in the connective tissue stroma, although electron probe X-ray analysis failed to disclose nickel-containing aggregates [68]. The retention of nickel suggests that it may be complexed by a ligand that avidly binds nickel, such as carnosine, as will be discussed later.

In contrast to cancers of the lung, which have been frequently reported in chromate-exposed workers, sinonasal cancers are relatively uncommon in these workers [69]. The first case of sinonasal cancer, reported in 1890 by Newman [70], was an adenocarcinoma of the inferior turbinate in a chrome pigment worker who also had perforated nasal septum. In total, 22 cases of sinonasal cancer have been documented in chromate-exposed workers throughout the world [70-75] (Table 3). An etiological role of Cr(VI) in these sinonasal cancers seems likely, although the evidence is not as strong as for nickel exposures [76-78]. Association of Cr(VI) exposure with increased risk of sinonasal cancer is supported by the observation of cellular atypia in brush biopsies of the rhinosinusal mucosa of chrome plating workers [79].

### Rhinotoxicity of Metals in Animals

Compounds of four metals, Ni, Cd, Cr, and Co, have been evaluated for rhinotoxicity in rodents after subacute or chronic exposures by inhalation (Table 4). The respiratory and olfactory epithelia of rodents are sensitive targets for toxicity from nickel sulfate and

Table 4. Nasal and olfactory toxicity of nickel, cadmium, chromium, and cobalt compounds in rodents.

Compound	Species	Exposures	Observations	Authors & yr
NiSO <sub>4</sub>	Rat	Ni inhalation (0.635 mg/m <sup>3</sup> , 6 hr/da, 16 da)	Atrophy of olfactory epithelium, loss of microvillar and sustentacular cells, and depletion of carnosine, but no evident impairment of olfactory function.	Miller et al [80] (1995); Evans et al [81] (1995)
NiSO <sub>4</sub> , αNi <sub>3</sub> S <sub>2</sub>	Rat, mouse	Ni inhalation (three graded levels for 16 da, 13 wk, or 2 yr)	Exposures to NiSO <sub>4</sub> and αNi <sub>3</sub> S <sub>2</sub> caused severe atrophy of the olfactory epithelium; αNi <sub>3</sub> S <sub>2</sub> exposures also caused chronic active inflammation of nasal tissues.	Nat Toxicology Program [82, 83] (1996)
CdO	Rat	Cd inhalation (0.25 or 0.5 mg/m <sup>3</sup> , 5 hr/da, 5 da/wk, 20 wk)	Exposures to CdO did not impair olfaction or cause histopathological changes, but Cd levels in the olfactory bulbs were much higher than controls.	Sun et al [84] (1996)
Na <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	Rat	Cr(VI) inhalation (0.2 mg/m <sup>3</sup> , 6 hr/da, 40 da)	Exposure to Cr(VI) produced no effects on nasal morphology; olfaction was not tested.	Hastings et al [85] (1994)
CoSO <sub>4</sub>	Rat, mouse	Co inhalation (graded levels (0.3-3.0 mg/m <sup>3</sup> , 6 hr/da, 5 da/wk, 2 yr)	Exposures to CoSO <sub>4</sub> caused hyperplasia and squamous metaplasia of nasal respiratory epithelium and atrophy and metaplasia of olfactory epithelium.	Bucher et al [86] (1999)

Table 5. Induction of anosmia in animals by intranasal administration of zinc solutions.

Compound	Animals	Exposure route	Observations	Authors & yr
ZnSO <sub>4</sub>	Rat	Nasal irrigation	The olfactory epithelium promptly degenerated after Zn(II) treatment; regeneration occurred after 7 days by proliferation of non-sensory ciliated cells.	Smith [90] (1938)
ZnSO <sub>4</sub>	Rat	Nasal irrigation	For 2-14 days after treatment, rats were unable to find buried, scented food pellets in an open field.	Alberts & Galef [91] (1971)
ZnSO <sub>4</sub>	Diverse	Nasal irrigation	Review of papers on ZnSO <sub>4</sub> induction of anosmia in the rat, mouse, hamster, gerbil, and sea turtle.	Alberts [92] (1974)
ZnSO <sub>4</sub>	Rat	Nasal irrigation	Treatment with ZnSO <sub>4</sub> produced severe interference in olfactory functions in rats trained in a wind-tunnel olfactometer to detect the presence of an odor or to discriminate odors of graded intensities; full recovery of odor intensity discrimination occurred in 8-10 days.	Slonick & Gutman [93] (1977)
ZnSO <sub>4</sub>	Mouse	Nasal irrigation	Behavioral studies demonstrated anosmia and histologic studies showed destruction of the olfactory epithelium.	Harding et al [94] (1978)
ZnSO <sub>4</sub>	Catfish	Nasal irrigation	Brief exposure to Zn(II) specifically destroyed olfactory receptor cells, while Cu(II), Fe(II), Hg(II), and Pb(II) had limited or no effects.	Cancalon [95] (1982)
ZnSO <sub>4</sub>	Rat (9 da old)	Nasal irrigation	At 1 day after treatment, pups showed deficit in odor-directed behavior; the behavioral deficit correlated with diminished 2-deoxyglucose uptake in olfactory bulbs, which is an index of odor-induced activity.	Stewart et al [96] (1983)
ZnSO <sub>4</sub>	Mouse	Nasal irrigation	Histological and electron microscopic studies showed sequential destruction of the olfactory epithelium, deafferentation and degeneration of the olfactory bulb, and slow, partial regeneration.	Burd [97] (1993)
ZnSO <sub>4</sub>	Rat	Nasal spray	After bilateral, but not unilateral, spraying, olfactory-dependent sniffing behavior was diminished.	Mayer & Rosenblatt [98] (1993)
ZnSO <sub>4</sub>	Homing pigeon	Unilateral nasal irrigation	In birds made anosmic by plugging the contralateral nostril, homing navigation was impaired, in comparison to control birds with an ipsilateral plugged nostril.	Benvenuti et al [99,100] (1992, 1996)

nickel subsulfide, which produce atrophy of the olfactory epithelium and, in the case of nickel subsulfide, chronic active inflammation of the nasal mucosa [82,83,87]. Despite severe epithelial atrophy, olfactory acuity was not demonstrably impaired in rats exposed for 16 days to inhalation of nickel sulfate particles [80,81]. Exposure of rats to cadmium oxide dust for 5 months did not impair olfactory acuity or induce histopathologic changes in the nasal mucosa, although the cadmium content of olfactory bulbs in CdO-exposed rats was much higher than in controls [84]. Exposure of rats to inhalation of sodium dichromate for 40 days had no evident effects on nasal morphology [85]. Exposure of rats and mice to cobalt sulfate for 2 years caused hyperplasia and squamous metaplasia of the respiratory epithelium, and atrophy and metaplasia of the olfactory epithelium [86].

#### Anosmia Induced by Zinc Sulfate

In 1938, nasal spraying with zinc sulfate solution ( $\text{ZnSO}_4$ , 1%, w/v) was tested in in the United States as a method of poliomyelitis prophylaxis in children [88,89]. The treatment proved ineffective in preventing polio, and some of the children developed transient or persistent anosmia [88,89].

Selected articles on the induction of anosmia in experimental animals by local exposure to zinc sulfate are listed in Table 5. Smith [90] found that the olfactory epithelium of rats promptly underwent degeneration following nasal irrigation with 1%  $\text{ZnSO}_4$  solution. Alberts and Galef [91] showed that nasal irrigation with 5%  $\text{ZnSO}_4$  solution transiently abolished the sense of smell in rats. After sham irrigation with saline, rats were able to locate buried, scented food pellets in an open field within 30 seconds, whereas after nasal irrigation with  $\text{ZnSO}_4$  solution, they were unable to locate pellets for at least 2 days and, in some cases, more than 2 weeks.

Since  $\text{ZnSO}_4$  treatment is more convenient than surgical ablation of the olfactory bulb, it has been widely adopted for experimental induction of anosmia in rodents, fishes, birds, and turtles [92]. Histological studies in mice by Harding et al [94] showed that nasal irrigation with  $\text{ZnSO}_4$  destroys the olfactory epithelium and causes secondary atrophy of the olfactory bulb. In catfish, Cancalon [95] found that

exposure to divalent Zn specifically destroyed olfactory receptor cells, while divalent salts of Cu, Fe, Hg, and Pb had limited or no effect. In  $\text{ZnSO}_4$ -treated mice, Burd [97] observed sequential destruction of the olfactory epithelium, deafferentation and degeneration of the olfactory bulb, and subsequent slow, partial regeneration.

Mayer and Rosenblatt [98] demonstrated that spraying with  $\text{ZnSO}_4$  solution must be bilateral in order to decrease olfactory-dependent sniffing behavior in rats. Benvenuti et al [99,100] induced impaired navigation of homing pigeons by irrigation of the nasal cavity with  $\text{ZnSO}_4$  solution. Following unilateral treatment with  $\text{ZnSO}_4$ , the homing navigation was unaffected if the ipsilateral nostril was plugged, but became severely impaired when the contralateral nostril was plugged [100].

#### Carnosine and Olfaction

In 1937, Margolis [101] and Neidle and Kandra [102] independently discovered the presence of unexpectedly high concentrations of carnosine ( $\beta$ -alanyl-L-histidine) in the mouse olfactory bulb. The concentration of carnosine was 10 to 50 times higher in the olfactory epithelium and olfactory bulb of normal mice, compared to other regions of the brain, and carnosine became undetectable in the olfactory epithelium and bulb after mice were rendered anosmic by  $\text{ZnSO}_4$  treatment [103]. Rapid and extensive incorporation of [ $^{14}\text{C}$ ]histidine into carnosine in intact bulbs suggested that carnosine is synthesized in the bulb by a specific enzyme, carnosine synthetase [102,104]. Surgical bulbectomy or peripheral deafferentation caused rapid, selective decline of carnosine content and carnosine synthetase activity in the olfactory epithelium or olfactory bulb of rodents [104,105].

In mice treated by intranasal irrigation with  $\text{ZnSO}_4$  solution, Harding et al [106] observed that anosmia was attended by long-term reduction of carnosine synthesis and transport in the primary olfactory pathway. Carnosine synthesis was virtually undetectable at two weeks after treatment, and even at one year after treatment did not exceed 10% of control values [106].

Studies on carnosine biochemistry are facilitated by the finding that one of the precursor amino acids,

$\beta$ -alanine, is incorporated specifically into carnosine and not into larger peptides and proteins [107]. Burd et al [108] administered  $\beta$ -[ $^3\text{H}$ ]alanine to the nasal cavity of hamsters and used biochemical techniques to identify the labeled compounds in the olfactory epithelium and olfactory bulb, as well as autoradiography to visualize uptake and transport of the label in primary afferents of the olfactory pathway. These studies demonstrated that carnosine becomes localized in peripheral olfactory axons and is rapidly transported via these axons to glomeruli of the olfactory bulb.

Ferriero and Margolis [104] proposed that carnosine functions as a neurotransmitter or neuromodulator in the olfactory pathway. MacLeod and Straughan [109] could not confirm this hypothesis by electrophysiological measurements after micro-iontophoretic application of carnosine to neurons in the rat olfactory bulb. On the other hand, studies of Kanaki et al [110] suggest that carnosine is an excitable neuroeffector between olfactory receptor neurons and olfactory bulb neurons, based on electrophysiological measurements of olfactory bulb neurons cultured by the organotypic slice technique. They noted inward current responses to carnosine that increased with increasing carnosine concentrations [110].

Immunohistochemical studies also suggest that carnosine may have a role in olfactory neurotransmission [111-113]. Carnosine-like immunoreactivity was shown by Sakai et al [111,112] to be localized specifically within the primary olfactory neuron and its axonic terminals in the glomerular layer of the rat olfactory bulb. In rats, monkeys, pigeons, and chickens, Biffò et al [113] found that olfactory receptor neurons, their axons, and their synapses in the olfactory bulb showed strongly positive immunoreactivity for carnosine, while elsewhere in the CNS, carnosine was evident only in glial cells.

Carnosine is known to bind Ni(II), Cd(II), Zn(II), Cu(II), and other metal ions in vitro, forming stable water-soluble complexes [114-118]. Datta et al [116] speculated that carnosine may play a role in nickel carcinogenesis, since the Ni(II)-carnosine complex activates oxygen and may thus promote the introduction of the mutagenic 8-hydroxy-2'-deoxyguanosine lesion into DNA [116].

Trombley et al [119] and Horning et al [120] provided evidence that carnosine influences synaptic

transmission in the olfactory bulb by modulating the inhibitory effects of Zn(II) and Cu(II) on *N*-methyl-D-aspartate (NMDA) and  $\gamma$ -aminobutyric acid (GABA) receptor-mediated currents [119,120]. Using whole-cell current- and voltage-clamp recording, they examined the direct and neuromodulatory actions of carnosine on rat olfactory bulb neurons in primary culture. Carnosine per se did not evoke a membrane current or affect the currents evoked by glutamate, GABA, or glycine, but Cu(II) and Zn(II) inhibited the NMDA and GABA receptor-mediated currents and inhibited synaptic transmission. Carnosine prevented the actions of Cu(II) and reduced the effects of Zn(II) [119]. Thus, carnosine may act in vivo to rescue neurons from Zn- and Cu-mediated neurotoxicity, serving as an endogenous neuroprotective agent [120].

Based on all of this evidence, the present author has suggested that carnosine may be involved in the neuronal uptake and translocation of metals from the olfactory epithelium to the olfactory bulb [121].

### Metal Transport via Olfactory Neurons

This topic has been reviewed by Tjälve and Henriksson [122]. The evidence for translocation of metals from the nose to the brain via olfactory pathways is summarized in Table 6. Several of these reports provide data for the velocities of axonal transport of metals along primary olfactory neurons, but the reports furnish little information about the physiological mechanisms and carrier molecules that are involved. Data on exposure-effect relationships for olfactory uptake of metals are lacking, since the exposures have generally involved a single high dosage of the test compound in aqueous solution or a particle suspension, either by intranasal administration or inhalation.

**Aluminum.** Rabbits that received nasal implants of gelfoam pads impregnated with soluble aluminum salts (aluminum lactate or aluminum chloride) developed granulomas in the olfactory bulb and cerebral cortex [123]. Rats that had subacute inhalation exposures to aluminum acetylacetonate accumulated Al in the olfactory bulb, pons-medulla, hippocampus, and cerebellum, as determined by fluorimetric analysis [124]. Rats that had subacute inhalation exposures to

Table 6. Metal uptake and translocation to the brain via olfactory pathways.

Metal	Compound	Species, route	Observations	Authors & yr
Al	Al-lactate AlCl <sub>3</sub>	Rabbit, nasal implants	Granulomas containing Al developed in olfactory bulbs and cerebral cortex following nasal implants of soluble Al compounds in gelfoam pads.	Perl & Good [123] (1987)
	Al-acetyl-acetate	Rat, inhalation, 3x/wk, 2 wk	Al was detected by morin fluorescence in the olfactory bulb, pons-medulla, hippocampus, and cerebellum.	Zatta et al [124] (1993)
	Al-chloro-hydrate	Rat, inhalation, 6 hr/da, 12 da	Positron-induced X-ray emission showed Al accumulation in the olfactory bulb.	Divine et al [125] (1999)
Cd	<sup>109</sup> CdCl <sub>2</sub>	Trout, added to aquarium water	Uptake of <sup>109</sup> Cd was documented in the olfactory rosette, olfactory nerve, and olfactory bulb.	Tjälve et al [126] (1986)
	<sup>109</sup> CdCl <sub>2</sub>	Pike, rat, unilateral intranasal application	Autoradiography and $\gamma$ -spectrometry showed <sup>109</sup> Cd in the ipsilateral olfactory bulb, but <sup>109</sup> Cd did not cross synapses to enter secondary olfactory neurons.	Gottofrey & Tjälve, [127] (1991), Tjälve et al [128] (1996)
	<sup>109</sup> CdCl <sub>2</sub>	Rat, intranasal instillation	After unilateral exposure, <sup>109</sup> Cd levels in the ipsilateral olfactory bulb were approximately 40x higher than in the contralateral bulb.	Hastings & Evans, [129] (1991), Evans & Hastings [130] (1992)
	<sup>109</sup> CdCl <sub>2</sub>	Rat, inhalation, 5 hr/day, 20 wk	After exposure, <sup>109</sup> Cd accumulated in olfactory bulbs, but olfactory sensation was unimpaired.	Sun et al [84] (1996)
Co	<sup>57</sup> CoCl <sub>2</sub>	Rat, intranasal instillation	<sup>57</sup> Co moved via olfactory nerve to olfactory bulb and some <sup>57</sup> Co was evident in secondary olfactory neurons.	Persson et al [131] (1998)
Hg	<sup>203</sup> HgCl <sub>2</sub>	Pike, intranasal application	<sup>203</sup> Hg moved via olfactory neurons to the ipsilateral olfactory bulb, but transfer to secondary olfactory neurons was not evident.	Borg-Neczak & Tjälve [132] (1996)
	<sup>203</sup> HgCl <sub>2</sub>	Rat, intranasal instillation	After 1 & 3 wk, the ipsilateral olfactory nerve and bulb contained more <sup>203</sup> Hg than the contralateral ones; no <sup>203</sup> Hg transport to secondary olfactory neurons was seen.	Henriksson & Tjälve [133] (1998)

Table 6 (continued). Metal uptake and translocation to the brain via olfactory pathways

Metal	Compound	Species, route	Observations	Authors & yr
Mn	$^{54}\text{MnCl}_2$	Trout, pike, rat, intranasal application	$^{54}\text{Mn}$ rapidly travelled along primary olfactory neurons, crossed synapses in the olfactory bulb, and reached large areas of the brain (and also the spinal cord in rats).	Rouleau et al [134] (1995), Tjälve et al [135] (1995), Henriksson et al [136] (1999)
	$\text{MnCl}_2$	Rat, intranasal instillation	After unilateral instillation, Mn reached peak levels in the ipsilateral olfactory bulb at 12 hr and stayed high for 3 days; after repeated instillations, Mn was also elevated in the ipsilateral striatum.	Gianutsos et al [137] (1997)
	$^{54}\text{MnCl}_2$	Rat, injection in olfactory bulb	One day after injection, $^{54}\text{Mn}$ was visualized in the ipsilateral piriform, amygdaloid, and entorhinal areas, indicating Mn transport along the olfactory tract to the olfactory cortex.	Takeda et al [138] (1998)
	$\text{MnCl}_2$	Rat, intranasal (1 to 3 weekly doses of 0, 10, 250, or 1000 $\mu\text{g}$ )	ELISA assays of glial fibrillary acidic protein (GFAP) and S-100b showed dose-related diminution of these astrocytic proteins in the olfactory cortex, thalamus, hypothalamus, and hippocampus, indicating that astrocytes are the initial targets of Mn toxicity in CNS.	Henriksson & Tjälve [139] (2000)
Ni	$^{63}\text{NiSO}_4$ , $^{63}\text{NiO}$	Rat, monkey, inhalation	At 2 to 20 wk after inhalation of soluble $^{63}\text{NiSO}_4$ particles, $^{63}\text{Ni}$ was seen in the olfactory bulbs. In contrast, after inhalation of insoluble $^{63}\text{NiO}$ , $^{63}\text{Ni}$ was not detected in the olfactory bulbs.	Lewis et al [141] (1994)
	$^{63}\text{NiCl}_2$	Rat, pike, intranasal application or instillation	Slow axonal transport of $^{63}\text{Ni}$ was observed via olfactory neurons to the olfactory bulb. The $^{63}\text{Ni}$ was bound to particulate and soluble constituents of the cytosol. $^{63}\text{Ni}$ was also evident in the olfactory peduncle, olfactory tubercle, and the cerebrum.	Henriksson et al, [142] (1997) Tallkvist et al [143] (1998)
Zn	$^{65}\text{ZnCl}_2$	Rat, direct injection in the olfactory bulb	At 24 hr post-injection, $^{65}\text{Zn}$ was seen in the ipsilateral piriform cortex, amygdaloid nuclei, and anterior commissure, consistent with $^{65}\text{Zn}$ transport along the olfactory tract.	Takeda et al [144] (1997)
	$^{65}\text{ZnCl}_2$	Rat, intranasal instillation	$^{65}\text{Zn}$ was transported via primary olfactory neurons to glomeruli in the olfactory bulb; slow uptake of $^{65}\text{Zn}$ into secondary olfactory neurons was also evident.	Persson et al [131] (1998)

aluminum chlorohydrate accumulated Al in the olfactory bulb, as assayed by positron-induced X-ray emission [125].

**Cadmium.** Tjälve and coworkers [126-128] and Evans and Hastings [129,130] have published strong evidence, based on autoradiography and  $\gamma$ -spectrometry, that  $^{109}\text{Cd}(\text{II})$  is transported along olfactory receptor neurons. In trout, pike, and rats,  $^{109}\text{Cd}$  was visualized in the olfactory receptor neurons and the olfactory bulb [126-128]. Following unilateral exposure of rats,  $^{109}\text{Cd}$  levels were approximately 40 times higher in the ipsilateral olfactory bulb, compared to the contralateral bulb [128].  $^{109}\text{Cd}$  did not cross the synapse in the olfactory bulb and enter secondary olfactory neurons.

In fish, the olfactory epithelium is a sensitive target for Cd toxicity, and olfactory sensation is critical for their survival. The olfactory nerve of the adult pike is particularly suited for studies of axoplasmic transport, since its axons are non-myelinated, uniform in diameter, and approximately 4.5 cm in length. Gottofrey and Tjälve [127] observed a well defined transport peak for  $^{109}\text{Cd}$  along the pike's olfactory axons, with an average velocity of approximately 2.4 mm/hr.

**Cobalt and mercury.** After intranasal exposure of rats to  $^{57}\text{CoCl}_2$  and intranasal exposure of pike and rats to  $^{203}\text{HgCl}_2$ , autoradiograms showed progressive uptake and translocation of  $^{57}\text{Co}$  and  $^{203}\text{Hg}$  via olfactory neurons to the olfactory bulb; traces of  $^{57}\text{Co}$  were transferred across the synapse to secondary olfactory neurons, but transfer of  $^{203}\text{Hg}$  to secondary neurons was not evident [132,133].

**Manganese.** After intranasal exposure of fishes and rats to  $^{54}\text{MnCl}_2$ , the  $^{54}\text{Mn}$  traveled rapidly along the primary olfactory neurons, crossed secondary and tertiary synapses, and eventually reached large areas of the brain, including the ipsilateral piriform, amygdaloid, and entorhinal areas [134-136]. In the pike, the peak of  $^{54}\text{Mn}$  radioactivity traveled along the olfactory axons with an average velocity of approximately 2.9 mm/hr [135]. Thus, dissemination of manganese via olfactory axons can occur rapidly, within hours or a few days. After examining this

evidence, Aschner et al [140] concluded that Mn may be transported along olfactory neurons and reach deeper brain structures under appropriate exposure conditions. However, the concentrations employed in the cited studies were much higher than those reported in human exposures, and additional studies were deemed necessary in order to establish that such mechanisms contribute to Mn accumulation in the CNS [140].

A recent report by Henriksson and Tjälve [139] provides biochemical evidence that Mn(II) uptake along the olfactory pathway causes neurotoxicity in rats. After intranasal instillation of  $\text{MnCl}_2$ , dose-related declines were noted in the concentrations of two astrocyte-specific proteins (ie, glial fibrillary acidic protein and S-100b protein) in the olfactory cortex, thalamus, hypothalamus, and hippocampus of rats. These findings are consistent with other evidence that astrocytes are targets of manganese toxicity in the CNS [139].

**Nickel.** Lewis et al [141] detected  $^{63}\text{Ni}$  in the olfactory bulbs of rats and monkeys following subacute inhalation exposure to particles containing soluble  $^{63}\text{NiSO}_4$ , but not after exposure to particles that contained insoluble  $^{63}\text{NiO}$ . Henriksson et al [142] and Tallquist et al [143] observed axonal transport of  $^{63}\text{Ni}$  from the nasal lumen via olfactory receptor neurons to the olfactory bulb, and low levels of  $^{63}\text{Ni}$  in the olfactory peduncle, olfactory tubercle, and even the cerebrum. In the olfactory nerve of the pike,  $^{63}\text{Ni}$  traveled along olfactory axons with an average velocity of 0.13 mm/hr, which falls in the category of slow axonal transport [143]. Thus, dissemination of nickel via olfactory axons evidently occurs slowly, over days or weeks.

**Zinc.** The translocation of  $^{65}\text{Zn}$  along olfactory pathways resembles that of  $^{63}\text{Ni}$ . Persson et al [131] reported that  $^{65}\text{Zn}$  travels along the axons of olfactory receptor neurons to their synapses in glomeruli of the olfactory bulb, and slowly crosses the synapses to enter secondary neurons. After injection of  $^{65}\text{Zn}$  directly into the olfactory bulb, Takeda et al [144] detected  $^{65}\text{Zn}$  in the ipsilateral piriform cortex, amygdaloid nuclei, and anterior commissure, consistent with Zn transport along the olfactory tract.

### Metal Abundance in the Olfactory Bulb

Studies of the relative abundance of metals in the olfactory bulb, compared to other regions of the brain, are summarized in Table 7. In untreated rats, Donaldson et al [145,146] found that, of 8 brain regions tested, Cu concentration was highest in the olfactory bulb, Zn highest in the olfactory bulb and hippocampus, and Mn highest in the hypothalamus and olfactory bulb. In two strains of untreated rats, Ono and Cherian [147] found that Zn and Cu concentrations were highest in the olfactory bulb, compared to the cortex, corpus striatum, hippocampus, thalamus plus hypothalamus, pons plus medulla oblongata, cerebellum, mid-brain, and white matter.

In untreated control rats, Domingo et al [148] observed that Al concentrations were much higher in the olfactory bulb than in other regions of the brain (cortex, hippocampus, striatum, cerebellum, thalamus, and rhachidical bulb). In untreated rats, Suzuki and Arito [150] and Clark et al [151] found that Cd concentrations were much higher in the olfactory bulb than in other regions of the brain.

In human brains sampled at autopsy, Maas et al [152] and Bonilla et al [153] found that Hg or Mn concentrations were abundant in the olfactory bulb, compared to other regions. From these observations, it appears that the olfactory bulb of untreated animals and humans contains elevated concentrations of certain metals, including Al, Cd, Cu, Hg, Mn, and Zn, in comparison to other parts of the brain. An important exception is lead, since Scheuhammer and Cherian [156] found that the mean Pb concentration in the olfactory bulb of untreated rats was comparable to the mean Pb concentration in residual areas of the brain.

Studies of animals treated with metal salts by supplementation of food or water, inhalation, or parenteral injection are also included in Table 7. At specified intervals after treatment, brain samples were analyzed to determine the regional distribution of the various metals. These studies showed that Al, Bi, Cd, Hg, and Mn were accumulated with greatest avidity by the olfactory bulb.

One factor that may contribute to localization of metals in the olfactory bulb, compared to other regions of the brain, is the passage of metals along the olfactory pathway, which has already been discussed. Another

factor may be the presence of metal-binding molecules, such as carnosine or metallothionein, in the olfactory bulb. Carnosine is exceptionally abundant in the olfactory bulb, as previously mentioned.

Metallothionein is also available, notably in astrocytes of the olfactory bulb cortex and in the glial cells that surround the bulb, as visualized in the dog brain by the immunohistochemical studies of Shimada et al [157]. Choudhuri et al [158] examined the constitutive expression of mRNAs for three metallothionein isoforms, MT-I, MT-II, and MT-III, in 7 regions of the mouse brain (olfactory bulb, cortex, caudate, hippocampus, thalamus, cerebellum, and brain stem). The olfactory bulb had the highest mRNA expression of all 3 isoforms [158].

Ono and Cherian [147] measured total metallothionein concentrations in 9 brain regions (olfactory bulb, cortex, corpus striatum, hippocampus, thalamus plus hypothalamus, pons plus medulla oblongata, cerebellum, midbrain, and white matter) in two rat strains. In Sprague-Dawley rats, no significant regional variations in brain metallothionein levels were observed except that the white matter showed the highest levels; in Lewis rats, metallothionein concentration was highest in the cerebral cortex and lowest in the olfactory bulb [147]. In neither strain was there any indication that metallothionein was responsible for the accumulation of copper and zinc in the olfactory bulb [147].

### Discussion

The toxic effects of chemicals on nasal tissues and olfactory sensation tend to be neglected in occupational and environmental medicine for several reasons: First, rhinoscopy of the anterior nasal passages with a speculum is not always performed during routine physical examinations, and endoscopic inspection of the nasal vault, ostiomeatal complex, and sphenoethmoidal recess requires special skills and equipment. Second, the odorant panel that is needed to test for hyposmia may be unavailable in the physician's office. Third, post-mortem examinations of nasal tissues (especially the olfactory neuro-epithelium) are infrequently performed, unless there is a specific clinical indication. The tendency of clinicians to disregard the nose and olfaction may be changing, because of technical advances in nasal

Table 7. Relative abundance or accumulation of metals in the olfactory bulb of the brain.

Metal	Species	Exposure	Observations	Authors & yr
Cu, Zn	Rat	None	Of 8 brain regions tested, Cu level was highest in the olfactory bulb; Zn level was highest in the hippocampus and next highest in the olfactory bulb.	Donaldson et al [145,146] (1973,1974)
Cu, Zn	Rat (2 strains)	None	Of 9 brain regions tested, Cu and Zn levels in the olfactory bulb were much higher than in the others.	Ono & Cherian [147] (1999)
Al	Rat	Al nitrate, po, 50 or 100 mg/kg/da, 6.5 mo	Al levels were highest in the olfactory bulb and lowest in the cortex and thalamus; Al retention in olfactory bulb was inversely related to the age of the rats.	Domingo et al [148] (1996)
Bi	Mouse	Bi subnitrate, ip, 2.5 g/kg	Bi levels were highest in the olfactory bulb and lowest in the cortex of 11 brain regions tested at 28 da.	Ross et al [149] (1994)
Cd	Rat	CdCl <sub>2</sub> , sc, 0.5 mg/kg/da, 25 wk	Marked Cd accumulation occurred in olfactory bulb of treated rats. In controls, Cd levels in the olfactory bulb were 2.6x higher than in the rest of the brain.	Suzuki & Arito [150] (1975)
Cd	Rat	Dietary Cd, 0.4, 20 or 100 µg/g, 67 da	Of 13 brain regions tested, Cd levels were highest in the olfactory bulb at all levels of dietary Cd intake.	Clark et al [151] (1985)
Hg	Human	None; 55 cases at autopsy	Mean Hg concentration in the olfactory bulb was 1.9x higher than in the occipital cortex.	Maas et al [152] (1996)
Hg	Rat	<sup>203</sup> HgCl <sub>2</sub> , ip	<sup>203</sup> Hg levels were higher in olfactory epithelium and bulb than in remaining brain at 1 & 3 wk post-dose.	Henriksson & Tjälve [133] (1998)
Mn	Rat	None	Of 8 brain regions tested, Mn levels were highest in the hypothalamus and next highest in the olfactory bulb.	Donaldson et al [146] (1974)
Mn	Human	None, 8 cases at autopsy	Of 39 brain regions tested, Mn levels were highest in the olfactory bulb and pineal gland.	Bonilla et al [153] (1982)
Mn	Mouse	MnCl <sub>2</sub> , ip, 5 mg/kg/da, 9 wk	Marked accumulation of Mn occurred in the olfactory bulb and striatum, which were the only brain regions tested.	Bonilla et al [154] (1994)
Mn	Rat	MnPO <sub>4</sub> in air, 0.3, 3.0 mg/m <sup>3</sup> , 6 h/da, 2 wk	Mn levels in the olfactory bulb were higher than in the two other regions of the brain that were tested (ie, striatum and cerebellum).	Vitarella et al [155] (2000)
Pb	Rat	None	Of 13 brain regions tested, Pb level was not elevated in olfactory bulb; it equalled the mean for whole brain.	Scheuhammer & Cherian [156] (1982)

endoscopy, improved imaging techniques based on magnetic resonance and computed tomography, and the introduction of convenient methods for olfactory testing, such as the University of Pennsylvania smell identification test (“UPSIT®” technique) [159] and the “Sniffin’Stick®” technique [160].

The sense of smell is often considered unimportant for humans, although it is obviously vital for animals, fishes, birds, and insects. There is increased recognition that olfaction alerts persons to dangers (fire, poisonous fumes, leaking gas, spoiled food, fecal contamination), affects appetite and nutrition (especially in the elderly), and contributes to psychological well-being and the quality of life (the appreciation of wines, foods, flowers, perfumes). Interest in olfaction has been stimulated by suggestions that hyposmia may be an early manifestation of Parkinson’s and Alzheimer’s diseases [161-164].

Based on the evidence that has been discussed, the author perceives several research needs: (a) to identify metal-binding constituents in nasal tissues, olfactory receptor neurons, and the olfactory bulb; (b) to elucidate the mechanisms of neuronal uptake and axonal transport of metals; (c) to delineate the dose-response and time-response relationships for translocation of metals via the olfactory pathway following exposure of animals to metal salts by inhalation; (d) to determine the incidence of olfactory impairment in workers exposed to metal-containing dusts, mists, and fumes (eg, welders, electroplaters, alkaline battery makers, refinery workers); and (e) to study the possible role of metal uptake via the olfactory system in the pathogenesis of Parkinson’s and Alzheimer’s diseases, as well as other neurodegenerative disorders [165-169].

The author urges practitioners of occupational and environmental medicine to be alert for the nasal toxicity of metals, to include the sense of smell in their review of each patient’s symptoms, to perform rhinoscopy during routine physical examinations, and to test selected patients for olfactory impairment.

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