Nickel exposure during sintering process and biomarkers of dose and renal effects

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Nickel absorption before and after measures to modernize a factory producing tools by means of metal dust sintering processes was studied. Nickel concentrations in the working environment and its potential absorption through the skin were monitored before and after a factory underwent modernization and compared with nickel excretion in urine. Kidney function was also assessed.
Nickel concentrations in the air during sintering processes were low before and after the factory was modernized, whereas nickel excretion in urine was near the allowable biological limit (ten times higher than the reference values) beforehand, while it quickly dropped to just twice the level in controls afterwards. No biochemical evidence of kidney involvement was found.
The high urinary nickel excretion during sintering processes despite the low environmental levels well demonstrates the role of the metal's absorption through the skin. Changes in the manufacturing procedures induces a marked decrease in the workers' nickel absorption. On the other hand, urinary nickel levels nearing the biological limit did not appear to have any effect on the workers' renal function.

Key words: metal dusts, nickel, sintering processes, kidney function, skin absorption

Lo scopo dello studio è stato quello di valutare l'inquinamento da nichel (Ni), l'esposizione professionale al metallo e il possibile coinvolgimento renale durante processi di sinterizzazione con polveri contenenti Ni in una azienda produttrice di oggettistica industriale. La sinterizzazione consiste in un trattamento termico di polveri o compatti ad una temperatura inferiore a quella di fusione del componente principale al fine di aumentarne la durezza per mutua coesione delle particelle. È stato effettuato un monitoraggio ambientale, determinando il contenuto di Ni nelle polveri aerodisperse e valutando il livello di contaminazione superficiale e cutaneo da Ni con wipe test e pads, ed un monitoraggio biologico dosando il Ni (NiU) nelle urine dei soggetti professionalmente esposti (29 addetti alla sinterizzazione e 20 ai trattamenti termici) e su un gruppo di controllo (26 addetti a uffici e pulizie); sono stati, inoltre, presi in considerazione alcuni indici renali di effetto quali le proteine totali, l'attività del N-acetil-β-D-glucusaminidasi e della Glutamina sintetasi. Il monitoraggio ambientale ha evidenziato livelli di Ni metallico in aria ben al di sotto del TLV-TWA, mentre è stata riscontrata una moderata ma costante presenza di Ni sulle superfici ambientali e cutanee monitorate. Il primo dosaggio del NiU ha dimostrato una significativa esposizione al metallo soprattutto negli addetti alla sinterizzazione, mentre i dosaggi successivi ad un intervento di bonifica hanno indicato una progressiva riduzione del NiU in tutti gli addetti; non si è comunque registrata alcuna alterazione della funzione renale. Nonostante il bassissimo livello di Ni in aria, è stata sufficiente la contaminazione della cute e delle superfici di lavoro per determinare una significativa esposizione dei lavoratori: il Ni veniva in parte ingerito, ma soprattutto assorbito per via cutanea grazie a condizioni igienico-comportamentali, tecniche e procedure lavorative inadeguate, indicanti una pressoché assente percezione del rischio; con il miglioramento delle condizioni di lavoro e l'adozione di dispositivi di protezione adeguati il problema è stato risolto.

Introduction

Nickel (Ni) is a metal widespread in nature and in working environments; its industrial uses are well defined, such as in the production of alloys, in welding or galvanic processes, for the production of Ni-Cd batteries, and so on [IARC, 1976; NIOSH, 1997]. A particular use of Ni is in sintering processes, in which hard metal dusts are compacted and undergo a heat treatment to produce industrial tools [Exner and Arzt, 1983].

Ni is a toxic metal and is known to cause allergic contact dermatitis [Oppel and Schnuch, 2006], even during the production and use of coins [Foti et al., 2005], as well as lung involvement, including asthma [Cruz et al., 2006], metal fume fever and, rarely, fibrosis [Newman, 1996] and renal damage [Vyskocil et al., 1994a; Vyskocil et al., 1994b].

It is also recognized as a carcinogen in the lung, nasal cavity and paranasal sinuses [Kasprzak et al., 2003; Verma et al., 2004], and is classified by the International Agency for Research on Cancer [IARC, 1990] in group 1 (carcinogenic to humans), in its highly soluble and insoluble compounds, and in 2B (possibly carcinogenic to humans) as metallic Ni. It is also considered genotoxic.

The aim of the present study was to assess the Ni pollution in the working environment, the occupational exposure and the possible effects on renal function of exposure to Ni during sintering processes involving hard metal dusts containing Ni. The exposure assessment was conducted before and after the factory underwent modernization.

Materials and Methods

Setting and Environmental monitoring

During sintering processes, metal dusts are placed in special moulds and compacted. The metal dusts are composed mainly of iron but also include Ni (3.6 - 4.4%), copper (1.35 - 1.65%), molybdenum (0.45 - 0.55%), a lubricant (0.63 - 0.77%) and graphite (0.17 - 0.23%). Zinc stearate is added to facilitate the release of the finished product from the moulds. Before the factory was modernized (2003-2004), these moulds were loaded by hand and the dust was removed using dusters or a jet of air; since modernization, the moulds have been loaded by an automated system, and the excess dust is removed automatically. The moulds are then loaded in the sintering furnace for heat treatment in a carbon-enriched atmosphere at 1120°C, a temperature below the melting point of the metals in the dust. The heat treatment is used to reduce the porosity between the particles and thus increase the mechanic strength of the tools.

Ni concentrations in the working environment were investigated before and after the factory was modernized. In particular, environmental monitoring had been done by others in the past; after the factory was modernized, five area samplers (sampling time about 2 hours) and five personal samplers (sampling time about 5 hours) were installed. Area sampling was done by means of conical inhalable dust samplers (Analitica s.n.c., Pesaro, Italy) using polyvinylchloride (PVC) membranes (20 mm in diameter) with a flux of 10 l·min^{-1}; personal sampling was done using IOM samplers (Analitica s.n.c., Pesaro, Italy) with PVC membranes (25 mm in diameter) with a flow rate of 2 l·min^{-1}. Total dust was determined by the gravimetric method conditioning the membranes before and after sampling for 48 hours at 20°C and 50% humidity, while Ni was measured by atomic absorption spectrometry after treating the membranes with ultra pure nitric acid.

After the factory had been modernized six wipe tests were used (15x15 cm surface area) on surfaces treated with Triton solution to test for dust dispersion on the surfaces. In addition, eight skin pads (5x10 cm surface area), treated as wipes, were applied to both forearms and legs to assess skin contamination in workers occupied at the automatic and semiautomatic presses. In a previous investigation, skin pads had been applied using the same procedure.

Subjects and Biological monitoring

Twenty-nine workers exposed to Ni during the sintering process and 20 exposed during the heat treatment were monitored by taking urine spot samples at the end of the working shift. The enrolment criterion was the availability of urinary Ni (NiU) test results prior to the factory's modernization. All subjects were Italian males of similar age (sintering: 39.1 ± 8.2 years old; heat treatment: 38.6 ± 6.7 years old). A control group (26 subjects) was enrolled from among the office staff and cleaners, who were matched for male gender and age (36.6 ± 9.7 years of age), geographical origin, and smoking habits.

NiU was determined at three different times: twice before the factory was modernized and once afterwards. The former analyses were performed by others, using atomic absorption spectrometry with the Zeeman effect, according to the Sunderman method [Sunderman, 1993]; the latter measurements were taken using an inductively coupled plasma mass spectrometry (ICP-MS, ELAN DRC II, Perkin Elmer, Waltham, USA) with a Dynamic Reaction Cell (DRC).

The urine samples were diluted with bidistilled water, using Tracepur® for inorganic trace analysis (Merck KgaA). The calibration standards were prepared with standard solutions of single elements ranging from 0.5 μg·l^{-1} to 10 μg·l^{-1} (Nickel in HNO$_3$ Atomic spectroscopy standard solution 1000 g·l^{-1}, Fluka). The sample solutions were pumped into the spray chamber with a peristaltic pump and the mass detected was 58 in the DRC with ammonia. The accuracy of the method was tested against the mean values obtained on certified reference materials, i.e. Environmental (control material 8 A/B) and Occupational (control material 2 A/B) G-EQUAS (German External Quality Assessment Scheme). The limit of detection (LOD) was 0.1 μg·l^{-1}, calculated as 3 standard deviations of the background signal obtained on 10 white samples.

Urinary values were adjusted to urinary creatinine concentration so that they could be compared with the previous measurements. For data collected after the modernization of the factory, the unadjusted NiU was also shown.

Biomarkers of renal effects were assessed only after the factory was modernized, measuring urinary total proteins (TUP) with the Pesce method [Pesce and Strande, 1973], urinary N-acetyl-ß-D-glucosaminidase (NAG) activity with the Lockwood method [Lockwood and Bosmann, 1979], and urinary glutamine synthetase (GS) activity with the Trevisan method [Trevisan et al., 1999].

Results and Discussion

Ni environmental pollution (Table 1) was very low after the factory was modernized, usually a hundred times lower than the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) of 1.5 mg·m^{-3} [ACGIH, 2010] such as for total dusts.

Table 1: Mean and standard deviation (SD) of levels of environmental monitoring at the sintering factory after its modernization (median and range in brackets)

type of monitoring	No. of samples	department/duty	total dust Mean mg·m^{-3}	total dust SD	Ni Mean mg·m^{-3}	Ni SD
area	5	sintering (manual)	0.101 (0.094) (0.056-0.153)	0.042	0.0067 (0.0062) (0.002-0.013)	0.0039
personal	2	sintering (manual)	(0.084-0.320)		(0.001-0.008)	
personal	1	sintering (automatic)	0.162		0.015	
personal	2	heat treatment	(0.130-0-248)		(0.001-0.0013)	
type of monitoring	**No. of samples**	**department/duty**			**Ni** **Mean** μg·cm^2	**SD**
wipe test	2	sintering (manual)			(0.13-0.46)	
	2	sintering (automatic)			(1.28-1.48)	
	2	changing rooms			(0.12-3.09)	
skin pads	4	sintering (manual)			2.15 (2.095) (1.33-3.08)	0.86
	4	sintering (automatic)			1.55 (1.505) (0.98-2.22)	0.67

Ni and dust pollution was also low before the factory was modernized (Table 2), while NiU (Figure 1) was as much as ten times the reference value (median 1.8 μg·l^{-1}; range 5th - 95th percentile

0.59 - 4.06 μg·l^{-1} [Goullé et al., 2005] in the sintering workers and about three times the reference value in the heat treatment workers.

In addition, skin pads applied before the factory was modernized showed severe Ni contamination (2.6 - 214 μg·cm^{-2}), which was much reduced after the factory's modernization (1.33 - 3.08 μg·cm^{-2}), when NiU levels in the heat treatment workers were comparable with those of the control group, and only twice as high in the sintering workers. Since the ACGIH does not provide a biological exposure index for NiU, the metal's excretion in the urine of exposed subjects was compared with the German biological threshold limit value (EKA), which is set at 45 μg·l^{-1} for an exposure to 0.5 mg·m^{-3}.

Table 2: Mean and standard deviation (SD) of levels of environmental monitoring at the sintering factory before its modernization (median and range in brackets)

type of monitoring	No. of samples	department/duty	total dust Mean mg·m^{-3}	total dust SD mg·m^{-3}	Ni Mean mg·m^{-3}	Ni SD mg·m^{-3}
area	4	sintering (manual)	0.15 (0.14) (0.10-0.23)	0.06	0.03 (0.02) (0.01-0.08)	0.03
personal	2	sintering (manual)	(0.42-1.41)		(<0.01-0.40)	
area	2	sintering (automatic)	(<0.10-0.39)		(0.02-0.15)	
personal	2	sintering (automatic)	(0.33-0.46)		(<0.01-0.03)	

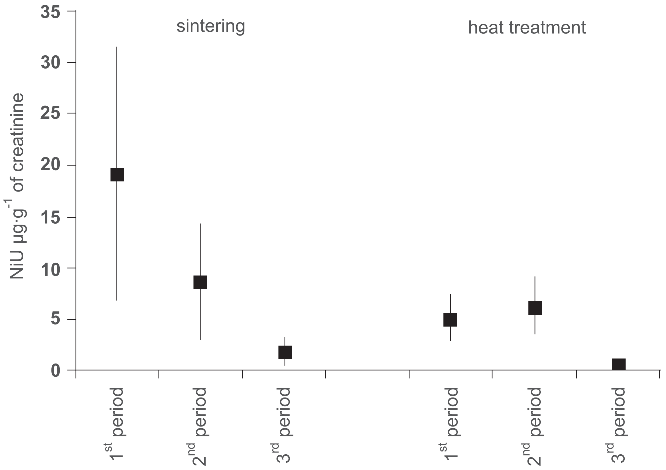

Figure 1: Ni excretion in urine (mean ± standard deviation) in sintering workers (left) and heat treatment workers (right) before (1st and 2nd period) and after the factory was modernized (3rd period)

After the factory was modernized, the NiU levels in the sintering workers (Table 3) was very low, nearing the upper limit of the reference values, even though higher than in controls and heat treatment workers (with statistically significant differences), owing to different working conditions that facilitated the metal's penetration of the skin.

Some Ni compounds, particularly the soluble compounds, can be actively absorbed through the skin [NIDI, 1997; Larese et al., 2007], and this type of absorption can explain high urinary Ni excretion in spite of low airborne Ni concentrations, as confirmed by the high Ni concentrations measured in pads collected before the factory was modernized.

A common confounding factor in measurements of NiU excretion is tobacco smoke, as are some foods, such as oats [Kristiansen et al., 1997]. Apparently, coin handling has no influence on urinary Ni excretion [Liden and Carter, 2001].

The aim of the factory's modernization was to improve the working conditions and reduce the risk of Ni absorption through the skin. That is why new standard procedures were adopted, such as more protective clothing, gloves and disposable dust-masks (FFP1 3 M 9310 model), automatic dispensers of devices and smoking was prohibited inside the factory. Rest rooms and changing rooms were reorganized to separate the clean from the soiled areas. These simple and inexpensive procedures considerably reduced the risk of Ni absorption.

Few studies are available on the nephrotoxic effects of Ni, but experimental investigations have shown changes in the glomeruli and tubules [Vyskocil et al., 1994b; Sato et al., 2005], and adverse effects of soluble Ni compounds on human tubular function in heavily-exposed workers at a chemical plant [Vyskocil et al., 1994a].

To monitor kidney function, we adopted a battery of tests, such as TUP, NAG and GS, that are routinely used at our laboratory to study the effects of xenobiotics on the kidney. NAG is a lysosomal enzyme largely used to detect proximal tubule damage caused by xenobiotics such as solvents [Brogren et al., 1986], metals [Ng et al., 1992] and silica [Lauwerys and Hoet, 2001]. The urinary detection of GS, a mitochondrial enzyme, has recently been recommended as a marker of S3 segment-specific damage in rats treated with hexachloro-1,3-butadiene [Trevisan et al., 1999].

Table 3: Mean and standard deviation (SD) of levels of biological exposure and renal effect indicators after factory modernization (median and range in brackets)

biomarkers	unit of measure	control subjects No. 26 Mean	control subjects No. 26 SD	sintering workers No. 29 Mean	sintering workers No. 29 SD	heat treatment workers No. 20 Mean	heat treatment workers No. 20 SD
NiU	μg·l^{-1}	1.77 (1.60) (0.1-5.7)	1.19	4.23 (3.60)[a,b] (0.1-12.7)	3.07	1.11 (0.90) (0.1-3.5)	0.78
NiU	μg/grams of creatinine	0.94 (0.90) (0.3-2.9)	0.51	1.80 (1.50)[c,d] (0.3-6.6)	1.40	0.55 (0.50) (0.1-1.8)	0.40
TUP	mg/mmol of creatinine	11.0 (8.3) (3.0-37.8)	8.2	9.2 (5.2) (1.1-78.9)	14.2	8.6 (6.7) (0.5-20.3)	5.9
NAG	μmol/mmol of creatinine	0.27 (0.27) (0.07-0.52)	0.11	0.29 (0.23) (0.05-1.08)	0.20	0.25 (0.23) (0.07-0.68)	0.14
GS	μmol/mmol of creatinine	0.68 (0.27) (0.00-4.64)	1.04	0.90 (0.52) (0.00-4.15)	1.09	0.90 (0.74) (0.00-2.56)	0.80

[a]p=0.0004 vs control subjects; [b]p<0.0001 vs heat treatment workers; [c]p=0.0039 vs control subjects; [d]p<0.0001 vs heat treatment workers (Mann-Whitney test).

Finally, TUP values are determined as general, non-specific indices of kidney involvement by chemicals.

The biochemical analyses (Table III) revealed no differences in renal function in the exposed workers by comparison with the controls, supporting evidence that Ni excretion in the urine below the level of 30 $\mu g \cdot g^{-1}$ of creatinine recommended in literature [Lauwerys and Hoet, 2001] is protective for the kidney.

In conclusion, despite the low Ni concentration in the working environment, urinary Ni excretion was high in our series of workers before their factory was modernized. Skin absorption was the likely reason for these high Ni excretion rates. Changes to the working procedures reduced the levels of Ni absorption. On the other hand, exposure to Ni causing urinary Ni excretion levels nearing the allowable limits does not damage the kidney.

References

American Conference of Governmental Industrial Hygienists., 2007. *Documentation of the Threshold Limit Values and Biological Exposures Indices*. Cincinnati (OH): ACGIH.

Brogren C.H., Christensen J.M., Rasmussen K., 1986. *Occupational exposure to chlorinated organic solvents and its effect on the renal excretion of N-acetyl-beta-D-glucosaminidase*. Arch. Toxicol., **9** (suppl.), 460-464.

Cruz M.J., Costa R., Marquilles E., Morell F., Munoz X., 2006. *Occupational asthma caused by chromium and nickel*. Arch. Bronconeumol., **42**, 302-306.

Exner H.E., Arzt E. Sintering processes. In Chan R.W., editor. Physical metallurgy. Amsterdam (North Holland): Pub Co, 3rd edition, 1983.

Foti C., Seidenari S., Antelmi A., Bonamonte D., Conserva A., Angelini G., 2005. *Provocative use test of 1 euro coin in nickel-sensitized subjects*. Contact Dermatitis, **52**, 167-168.

Goullé J.P., Mahieu L., Castermant J., Neveu N., Bonneau L., Lainé G., Bouige D., Lacroix C., 2005. *Metal and metalloid multi-elementary ICP-MS validation in whole blood, plasma, urine and hair. Reference values*. Forensic Sci. Int., **153**, 39-44.

International Agency for Research on Cancer. *Monographs on the evaluation of carcinogenic risk to humans. Cadmium, Nickel, some Epoxides, Miscellaneous Industrial Chemicals, and General Considerations on Volatile Anesthetics*. Lyon (France): IARC, vol. 11, 1976.

International Agency for Research on Cancer. *Monographs on the evaluation of carcinogenic risk to humans, Chromium, Nickel and welding*. Lyon (France): IARC, vol. 49, 1990.

Kasprzak K.S., Sunderman F.W. Jr., Salnikow K., 2003. *Nickel carcinogenesis*. Mut. Res. (Fundam. Mol. Mech. Mut.), **533**, 67-97.

Kristiansen J., Christensen J.M., Iversen B.S., 1997. *Toxic trace element reference levels in blood and urine: influence of gender and lifestyle factors*. Sci. Total Environ., **204**, 147-160.

Larese F., Adami G., Venier M., Maina G., Renzi N., 2007. *In vitro percutaneous absorption of metal compounds*. Toxicol. Lett., 170, 49-56.

Lauwerys R.R., Hoet P., *Industrial Chemical Exposure: Guidelines for Biological Monitoring*. London (England): Lewis Publishers; 3rd edition, 2001.

Liden C., Carter S., 2001. *Nickel release from coins*. Contact Dermatitis, **44**, 160-165.

Lockwood T.D., Bosmann H.B., 1979. *The use of urinary N-acetyl-beta-glucosaminidase in human renal toxicology. I. Partial biochemical characterization and excretion in humans and release from the isolated perfused rat kidney*. Toxicol. Appl. Pharmacol., **49**, 323-336.

Meyer B.R., Fischbein A., Rosenman K., Lerman Y., Drayer D.E., Reidenberg M.M., 1984. *Increased urinary enzyme excretion in workers exposed to nephrotoxic chemicals*. Am. J. Med., **76**, 989-998.

National Institute for Occupational Safety and Health. *Criteria for a Recommended Standard. Occupational exposure to inorganic Nickel*. Washington (DC): US Department of Health Education and Welfare [DHEW (NIOSH) Publication No: 77-164], 1997.

Newman L. Metals. In Haber P., Schenker M.B. Balmes J., editors. Occupational and environmental respiratory disease. St. Louis (MO): Mosby; 1st edition, 1996.

Ng T.P., Ng Y.L., Lee H.S., Chia K.S., Ong H.Y., 1992. *A study of silica nephrotoxicity in exposed silicotic and non-silicotic workers*. Brit. J. Ind. Med., **49**, 35-37.

NIDI. Safe use of Nickel in the Workplace. Canada: Nickel Development Institute; 2nd edition, 1997.

Oppel T., Schnuch A., 2006. T*he most frequent allergens in allergic contact dermatitis*. Dtsch. Med. Wochenschr., **131**, 1584-1589.

Pesce M.A., Strande C.S., 1973. *A new micromethod for determination of protein in cerebrospinal fluid and urine*. Clin. Chem., **19**, 1265-1267.

Sato K., Kusaka Y., Suganuma N., Akino H., Yokoyama O., 2005. *Direct effect of* CoC$_{12}$ *and* NiCl$_2$ *on citrate uptake by the rat renal brush border membrane*. Ind. Health, **43**, 574-579.

Sunderman F.W., 1993. *Biological monitoring of nickel in humans*. Scand. J. Work Environ. Health, **19**, 34-38.

Trevisan A., Cristofori P., Fanelli G., 1999. *Glutamine synthetase activity in rat urine as sensitive marker to detect S3 segment-specific injury of proximal tubule induced by xenobiotics*. Arch. Toxicol., **73**, 445-449.

Verma R., Ramnath J., Clemens F., Kaspin L.C., Landolph J.R., 2004. *Molecular biology of nickel carcinogenesis: identification of differentially expressed genes in morphologically transformed C3H10T1/2Cl 8 mouse embryo fibroblast cell lines induced by specific insoluble nickel compounds*. Mol. Cell. Biochem., **255**, 203-216.

Vyskocil A., Senft V., Viau C., Cizkova M., Kohout J., 1994a. *Biochemical renal changes in workers exposed to soluble nickel compounds*. Hum. Exp. Toxicol., **13**, 257-261.

Vyskocil A., Viau C., Cizkova M., 1994b. *Chronic nephrotoxicity of soluble nickel in rats*. Hum. Exp. Toxicol., **13**, 689-693.