

# Multi-component assessment of worker exposures in a copper refinery

## Part 2. Biological exposure indices for copper, nickel and cobalt†

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Urinary copper (Cu), nickel (Ni) and cobalt (Co) concentrations were determined for 127 Cu refinery workers (40 females, 87 males), with values of the 95% upper confidence interval of the geometric mean in nmol per mmol creatinine of 89 (Ni), 42 (Cu) and 3.4 (Co) for electrorefinery workers. In the pyrometallurgical departments, the corresponding concentrations were 37 (Ni), 99 (Cu) and 11 (Co). Female workers had higher Co urinary concentrations than males ( $p \leq 0.05$ ) while no gender difference was observed for Cu and Ni. Inter-elemental correlations were moderate to weak. Based on the inhalable aerosol levels reported previously for the same workers, the observed urinary Cu concentrations were considerably lower than expected, relative to Co and Ni. This is interpreted in terms of the current understanding of Cu homeostasis.

### Introduction

In a previous paper,<sup>1</sup> we focused on the air exposure levels of major workplace contaminants in the Cu production department of the Ni refinery complex located in Monchegorsk, Kola Peninsula, Murmansk Region, Russia. This exposure assessment of refinery workers was conducted as a component of a reproductive health study among both female and male Ni workers.<sup>2</sup> Compared to international occupational exposure levels (OELs), the workplace air concentrations of Cu and Ni reported indicated that these exposures warrant consideration in this context. Based on the ratio of the geometric means of inhalable Cu and Ni concentrations, exposures were 13-fold (pyrometallurgical workers) and 2.3-fold (electrorefinery workers) higher in Cu than Ni; Co concentrations were more than 20-fold lower than those for Ni. The focal point of the present paper will be on biological indices of exposures to Co, Cu and Ni.

A few previous studies have examined occupational exposure to Cu among workers,<sup>3–5</sup> but they are limited in scope. Very few publications have addressed the relationship between air measurements and Cu concentrations in body fluids. In one study,<sup>6</sup> serum Cu concentrations in smelter workers were unrelated to occupational exposure in the range of 0.12 to 17.6 mg m<sup>-3</sup> (personal air samples).

Unlike Ni, Cu is an essential element of which humans need a substantial daily intake to maintain normal body functions.<sup>7</sup> Cu in serum or urine, the Cu-transport protein ceruloplasmin and the enzyme activity of erythrocyte superoxide dismutase (SOD), are often used as indices of Cu status.<sup>8,9</sup> It appears that these measures respond in individuals deficient in Cu, by contrast to those with normal Cu levels, even when taking Cu supplements.<sup>10–12</sup> Tracer studies have shown that endogenous Cu excretion into bile is a major point of regulation of the body's Cu stores, as is regulation of gastrointestinal absorption. Relative absorption appears to be more efficient with lower dietary Cu intake; conversely, biliary excretion seems to increase when dietary intake is enhanced.<sup>13,14</sup> Interestingly, in terms of toxicity, Cu is relatively non-toxic<sup>15</sup> by comparison to Co<sup>16</sup> and Ni.<sup>17</sup> Metal fume fever has been reported in workers exposed to Cu oxide, metallic dust or fumes and is a 24–48 h illness characterised by chills, fever, aching muscles, dryness in the mouth and throat, and headache.<sup>15</sup> However, Borak *et al.*<sup>18</sup> have challenged the evidence for a causal relationship with Cu fume exposure based on a critical review of reported cases. Exposures to Cu-containing dusts and mists have also been associated with respiratory irritation, including coughing, sneezing, thoracic pain, runny nose and possibly mucosal tissue alterations.<sup>15</sup>

One of the objectives of this work is to establish relationships between air and urinary concentrations of Co, Cu and Ni in Cu-electrorefinery and Cu-smelter workers. Others are to explore the occurrence of inter-metal relationships for urinary measurements and to evaluate Cu excretion in urine as a biological exposure index of Cu.

### Experimental

#### Process and job descriptions

Details about the Cu refining process and associated worker job descriptions were provided in our earlier report of the Cu

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**Table 1** Urinary Ni concentrations for pyrometallurgical workers at the Monchegorsk copper refinery

Job category	Ni/creatinine (nmol per mmol) <sup>a,b</sup>				Number of urine samples	Number of workers: total (females)
	Mean ± s	Median	Geometric mean	95% CI		
Anode casting furnace: crane operator	6.5 ± 2.7	5.5	6.1	4.1–8.9	23	5 (5)
Anode casting furnace: pouring worker	12 ± 10	8.3	9.2	5.3–16	18	6 (0)
Anode casting furnace: smelter	12 ± 4.6	9.7	11	8.3–15	28	7 (0)
Convertor furnace: crane operator	12 ± 12	7.8	9.5	6.0–15	39	10 (2)
Convertor worker	14 ± 4.1	16	14	11–17	45	13 (0)
Crane operator assistant on the floor; anode casting + reverberatory furnaces (smelter main passage)	16 ± 6.0	16	15	10–22	24	5 (0)
Cu concentrate loader (feeder)	27 ± 11	26	24	17–37	23	5 (5)
Filter worker	22 ± 10	22	19	10–32	25	6 (5)
Reverberatory furnace, smelter	15 ± 8.3	12	13	10–17	30	10 (0)
Others (gas scrubbing; laboratory chemistry worker; tractor driver; anode casting and reverberatory furnaces; quality control)	6.8 ± 2.7	7.3	6.5	4.1–10	12	4 (1)
All female workers	17 ± 12	16	13	8.9–19	77	18
All male workers	13 ± 7.8	12	12	10–13	190	53
All	14 ± 9.0	12	12	10–14	267	71

<sup>a</sup> The data points used for the statistical calculations corresponded to the average of the 3 or more spot urine samples collected from each individual worker (see text). <sup>b</sup> An appropriate conversion factor for nmol per mmol creat. to  $\mu\text{g L}^{-1}$  is 0.69, since 1 mol of creatinine is equal to 85 L based on the observation that on average (*i.e.*, on a group basis) 1 L of urine contains 1.33 g of creatinine;<sup>21,22</sup> the factor is equal to  $\text{AW}_{\text{Ni}}/85$ .

levels in the inhalable, thoracic and respirable workplace aerosol particle-size fractions.<sup>1</sup>

### Urine samples

Urine specimens were collected at home, to minimise inadvertent contamination, directly into a clean plastic cup for transfer to containers (Universal Container, volume 25 mL, Nalge Nunc Int. Corp., Rochester, NY, USA), which were tested and found not to contaminate the urine samples (*i.e.*, below the detection limits, DL). The urine samples corresponded to the first-voided urine after the shift and/or the morning after the shift or its equivalent, for up to 5 shifts. The timing coincided with the personal air sampling for 2 to 4 shifts as described in Part 1.<sup>1</sup> The urine samples were transported in doubly-sealed containers simultaneously (at ambient temperature) to NIOH in Oslo and the Public Health Research Institute in St. Petersburg within 3 d of collection; on arrival they were frozen and stored at  $-20\text{ }^{\circ}\text{C}$ . As observed previously,<sup>19</sup> there was no evidence of specimen integrity loss.

To prevent any risk of laboratory acquired infections and to dissolve urine precipitates, all urine samples were heated for one hour at  $95\text{ }^{\circ}\text{C}$  prior to analysis. Ni, Co and Cu were measured by electrothermal atomic absorption spectrometry using a Perkin–Elmer SIMAA 6000–THGA System and a Hitachi Z-7000 spectrometer. Calibration was achieved with matched standard-urine solutions. Human urine Seronorm™ trace element quality control material (STE Batch 403125, Sero Ltd, Asker, Norway) was employed throughout to monitor the accuracy and reproducibility of the measurements. The day-to-day variations of the Ni, Co and Cu measurements in this quality control material were typically 7, 8 and 7%, respectively. The concentrations measured were in good agreement with the values reported by the manufacturer: Ni found  $38.9 \pm 2.7\text{ }\mu\text{g L}^{-1}$  ( $n = 152$ ), expected  $40\text{ }\mu\text{g L}^{-1}$ ; Co found  $10.5 \pm 0.9\text{ }\mu\text{g L}^{-1}$  ( $n = 118$ ), expected  $10\text{ }\mu\text{g L}^{-1}$ ; Cu found  $28 \pm 1.8\text{ }\mu\text{g L}^{-1}$  ( $n = 138$ ), expected  $28\text{ }\mu\text{g L}^{-1}$ .

The detection limit (DL;  $3 \times$  standard deviation,  $s$ , of the blank) was 0.9, 0.5 and  $0.5\text{ }\mu\text{g L}^{-1}$  for Ni, Co and Cu, respectively. Creatinine (creat.) was measured in urine by a Beckman creatinine analyser (Beckman Instruments, Brea, CA, USA) based on the Jaffe reaction.<sup>20</sup>

### Statistical methods

Homogeneity of variance indicated that the air and urinary metal concentrations had a log-normal distribution. Geometric means were tested for differences at the 95% level by a one-way analysis of variance with unequal replications using the  $F$ -statistic. The median, arithmetic mean  $\pm$  standard deviation, the geometric mean and its 95% confidence interval (CI) are provided to facilitate the discussion of the data. In the linear regression analyses, an association was accepted when the 95% CI of the regression coefficient did not include zero. Even though large variability in exposure and urinary metal levels were observed between workers, working shifts, job categories and departments, all data points were given equal statistical weight in the calculations. One half of the DL value was substituted for the few results below this concentration.

The study was approved by the Regional Research-Ethics Board of the University of Tromsø, the McMaster University Research-Ethics Board and the Norwegian Data Inspectorate.

### Results

It is clear from the data presented in Tables 1–4 (also refer to Fig. 1 and 2) that the ranges of urinary concentrations for Ni, Cu and Co were quite different, with values of the 95% upper confidence interval of the geometric mean in nmol per mmol creat. at 87 (Ni), 42 (Cu) and 3.4 (Co; data not shown) for electrorefinery workers; for the pyrometallurgical workers, the corresponding concentrations were 37 (Ni), 99 (Cu) and 11

**Table 2** Urinary Ni concentrations for electrorefinery workers at the Monchegorsk copper refinery

Job category	Ni/creatinine (nmol per mmol) <sup>a,b</sup>				Number of urine samples	Number of workers: total (females)
	Mean ± s	Median	Geometric mean	95% CI		
Cathode worker	46 ± 32	44	36	14–87	10	4 (0)
Crane operator	31 ± 13	24	27	22–34	44	11 (11)
Electrolysis worker	51 ± 58	31	36	22–58	34	11 (0)
Electrolyte cycle worker	34 ± 9.5	32	34	27–43	33	5 (5)
Loader–unloader	20 ± 8.0	17	19	15–24	31	9 (0)
Ni sulfate worker	34 ± 19	31	29	19–46	24	6 (1)
Slime worker	32 ± 5.8	32	31	26–37	27	4 (4)
Others (shipping preparation: loader of cathodes; electrician; riveting of cathodes; Cu removal)	19 ± 7.3	20	17	12–26	17	6 (1)
All female workers	31 ± 10	27	29	26–34	108	22
All male workers	36 ± 37	24	26	20–34	112	34
All	34 ± 31	27	27	24–32	220	56

<sup>a</sup> The data points used for the statistical calculations corresponded to the average of the 3 or more spot urine samples collected from each individual worker (see text). <sup>b</sup> For the conversion factor to  $\mu\text{g L}^{-1}$  see footnote b, Table 1.

(Co) nmol per mmol creat. Based on geometric mean concentrations, the urinary excretions of Cu and Co were not different between the electrorefinery and smelter workers ( $p > 0.05$ ; all workers), but with higher values for Ni among the electrorefinery workers. Only for Co were there any gender differences, with females having higher Co urinary levels than males in both the electrorefinery ( $p \leq 0.05$ ) and the smelter ( $p \leq 0.05$ ). The highest geometric mean urinary concentration for all 3 metals occurred in the smelter for Cu concentrate loaders and filter workers, while in the electrorefinery the observed levels were more uniform. The relationship between urinary Cu and urinary Ni plotted in Fig. 1 ( $p \leq 0.001$ ) and that for Ni and Co in Fig. 2 ( $p < 0.001$ ), suggest moderate associations between the excretion of these metals.

As illustrated in Fig. 3 and 4 and for Cu and Ni, respectively, the relationships between air and urine concentrations

for the Cu refinery workers appears complex and were not statistically significant ( $p > 0.05$ ); the same observation applies to Co (data not shown).

## Discussion

Taking the urinary background level of  $85 \text{ nmol L}^{-1}$  of Ni reported for areas of the Kola Peninsula with Ni-refineries<sup>23</sup> suggests, by reference to the regression equations in Fig. 1, that the corresponding value for Cu is around  $165 \text{ nmol L}^{-1}$  ( $10.5 \mu\text{g L}^{-1}$  or  $14.0 \text{ nmol per mmol creat.}$ ). This concentration is consistent with those reported in other recent international studies.<sup>8,24–26</sup> It is evident from the plots in Fig. 1 that, on average, the increase in urinary Ni concentrations above background are consistently at least 3-fold higher compared to Cu. This is unexpected considering that the water-soluble and

**Table 3** Urinary Cu concentrations for pyrometallurgical workers at the Monchegorsk copper refinery

Job category	Cu/creatinine (nmol per mmol) <sup>a,b</sup>				Number of urine samples	Number of workers: total (females)
	Mean ± s	Median	Geometric mean	95% CI		
Anode casting furnace: crane operator	20 ± 8.3	20	19	14–28	23	5 (5)
Anode casting furnace: pouring worker	17 ± 9.4	15	16	11–24	18	6 (0)
Anode casting furnace: smelter	19 ± 8.8	17	17	12–24	28	7 (0)
Convertor furnace: crane operator	39 ± 60	16	24	14–41	39	10 (2)
Convertor worker	28 ± 44	16	19	13–28	45	13 (0)
Crane operator assistant at the floor; anode casting + reverberatory furnaces (smelter main passage)	25 ± 12	20	22	16–35	24	5 (0)
Cu concentrate loader (feeder)	63 ± 61	47	46	20–99	23	5 (5)
Filter worker	47 ± 58	25	30	14–63	25	6 (5)
Reverberatory furnace, smelter	16 ± 5.8	16	16	13–19	30	10 (0)
Others (gas scrubbing; laboratory chemistry worker; tractor driver; anode casting and reverberatory furnaces; quality control)	16 ± 8.2	14	16	9.6–24	12	4 (1)
All female workers	41 ± 47	24	28	19–41	77	18
All male workers	25 ± 35	16	19	16–22	190	53
All	28 ± 38	17	20	17–24	267	71

<sup>a</sup> The data points used for the statistical calculations corresponded to the average of the 3 or more spot urine samples collected from each individual worker (see text). <sup>b</sup> An appropriate conversion factor for nmol per mmol creat. to  $\mu\text{g L}^{-1}$  is 0.75, since 1 mol of creatinine is equal to 85 L based on the observation that on average (*i.e.*, on a group basis) 1 L of urine contains 1.33 g of creatinine;<sup>21,22</sup> the factor is equal to  $\text{AW}_{\text{Cu}}/85$ .

**Table 4** Urinary Cu concentrations for electrorefining workers at the Monchegorsk copper refinery

Job category	Cu/creatinine (nmol per mmol) <sup>a,b</sup>				Number of urine samples	Number of workers: total (females)
	Mean ± s	Median	Geometric mean	95% CI		
Cathode worker	25 ± 5.5	25	25	20–31	10	4 (0)
Crane operator	20 ± 6.9	20	19	16–24	44	11 (11)
Electrolysis worker on duty	24 ± 9.0	22	22	17–27	34	11 (0)
Electrolyte cycle	19 ± 3.1	19	19	16–22	33	5 (5)
Loader–unloader	24 ± 13	17	20	14–28	31	9 (0)
Ni sulfate worker	28 ± 19	17	24	15–38	24	6 (1)
Slime worker	16 ± 2.4	16	16	13–17	27	4 (4)
Others (shipping preparation: loader of cathodes; electrician; riveting of cathodes; Cu removal)	30 ± 16	28	25	14–42	17	6 (1)
All female workers	20 ± 6.8	19	19	17–22	108	22
All male workers	25 ± 13	22	22	19–27	112	34
All	24 ± 11	19	20	19–24	220	56

<sup>a</sup> The data points used for the statistical calculations corresponded to the average of the 3 or more spot urine samples collected from each individual worker (see text). <sup>b</sup> For the conversion factor to  $\mu\text{g L}^{-1}$ , see footnote b, Table 3.

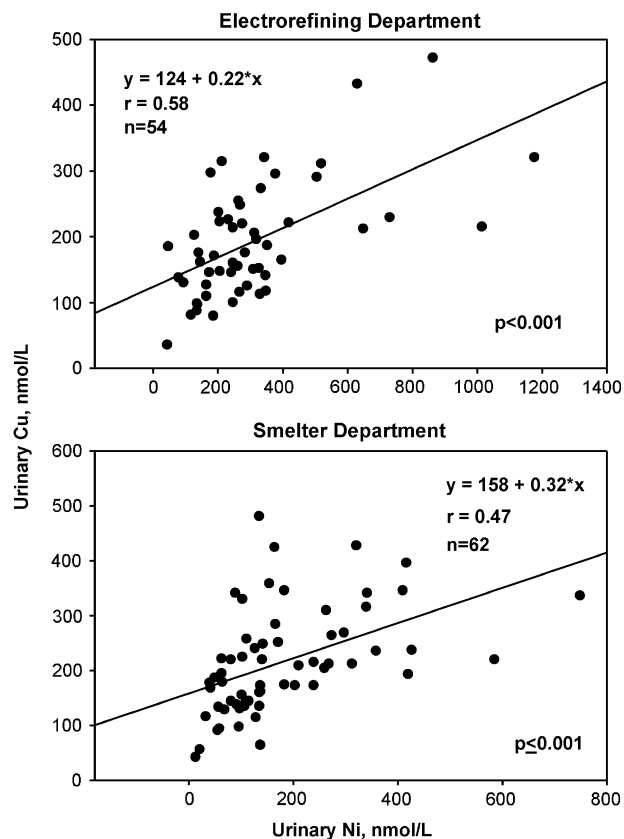
insoluble Cu aerosol subfractions were, respectively, 19- and 12.5-fold higher than the corresponding Ni values.<sup>1</sup>

The lack of a clean relationship between the water-soluble subfractions of Ni and Co and the corresponding urine concentrations, and for that matter for the inhalable aerosol fraction (data not shown), suggests that it is difficult to predict urinary concentrations of these metals on the basis of air measurements. However, applying the historic and somewhat generalised relationship for group Ni measurements shown

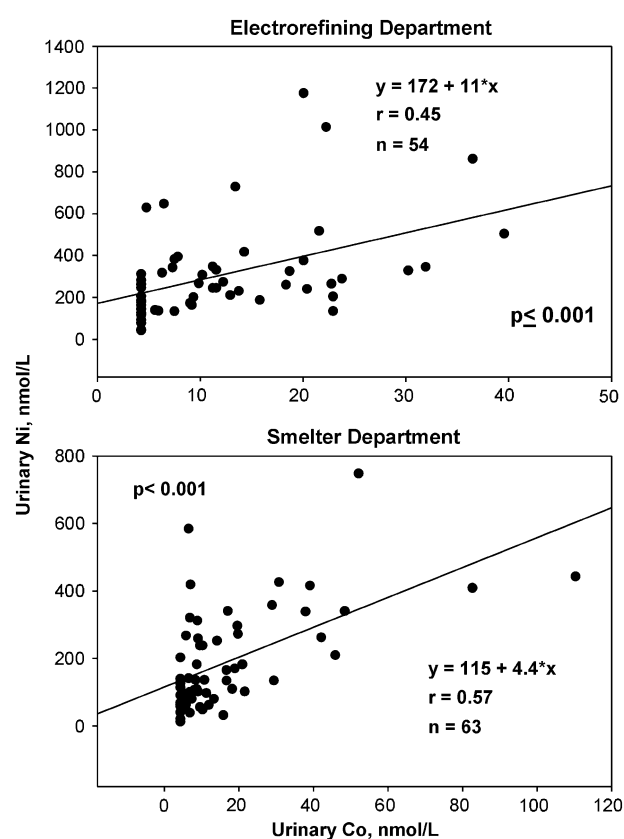
below<sup>19,21</sup> to the arithmetic means and standard deviations of the observed inhalable nickel aerosol fractions in Table 1 and 2 is quite helpful.

$$U_{\text{Ni}} \text{ (nmol per mmol creat.)} = 500 \text{ Air}_{\text{Ni}} \text{ (inhalable, mg m}^{-3}\text{)} + 7 \text{ (electrolytic refinery workers)}$$

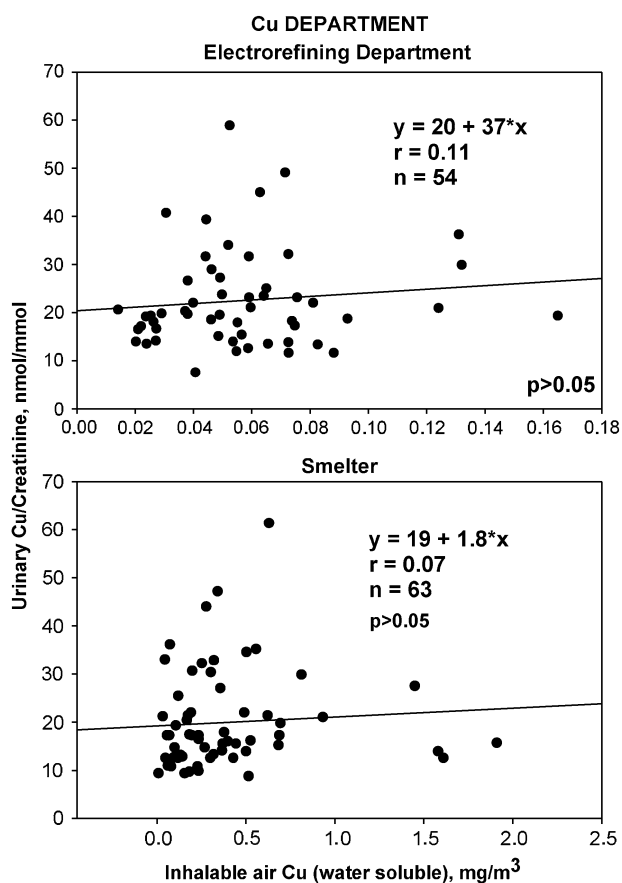
$$U_{\text{Ni}} \text{ (nmol per mmol creat.)} = 60 \text{ Air}_{\text{Ni}} \text{ (inhalable, mg m}^{-3}\text{)} + 7 \text{ (pyrometallurgical workers)}$$



**Fig. 1** Plots of urinary Cu versus urinary Ni concentrations for workers in the electrorefining and smelter departments.



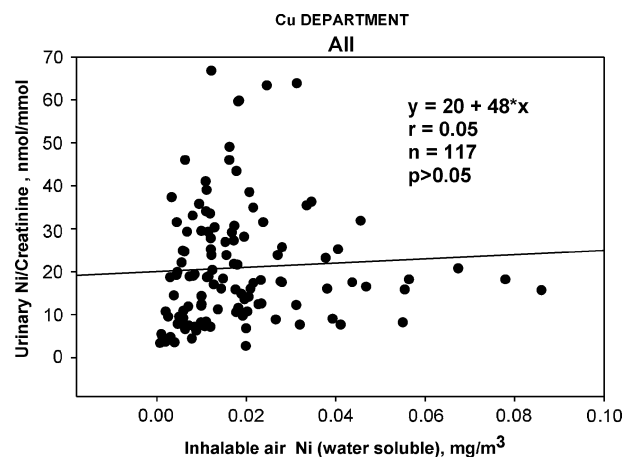
**Fig. 2** Plots of urinary Ni versus urinary Co concentrations for workers in the electrorefining and smelter departments.



**Fig. 3** Plots of urinary Cu versus the inhalable water-soluble Cu subfraction for workers in the electrorefinery and smelter departments.

These formulations take into account that for Norwegian nickel refinery workers it has been established that, on average, the inhalable fraction is nearly twice the corresponding “total” aerosol measurements,<sup>27</sup> as well as the observation that near Ni-refineries in the Kola Peninsula, the background urine Ni concentration for non-occupationally exposed individuals is 7 nmol per mmol creat.<sup>23</sup> On this basis, the observed and predicted urinary Ni concentrations are  $34 \pm 31$  nmol per mmol creat. and  $27 \pm 31$  nmol per mmol creat., respectively, for electrorefinery workers; and similarly,  $18 \pm 9$  and  $14 \pm 9$  nmol per mmol creat. for pyrometallurgical workers.

The fact that the urinary Cu concentrations are considerably lower than expected relative to Co and Ni can be understood in terms of the unique metabolism of Cu. While Co and Ni are primarily excreted in the urine,<sup>19,20</sup> elimination into bile is predominant for Cu.<sup>14,28,29</sup> Only about 5% of absorbed Cu appears in the urine. As noted in the introduction, Cu homeostasis is achieved by regulating Cu absorption in the gastrointestinal tract and its excretion into bile. Cu appears to cross the intestinal absorptive cell (enterocyte) plasma membrane in two ways, namely by way of the human Cu transporter 1 (Ctr1) and the divalent metal transporter 1 (DMT-1).<sup>30</sup> The former is the default pathway for Cu, while the latter constitutes the major uptake pathway for iron (as  $\text{Fe}^{2+}$ ) and  $\text{Mn}^{2+}$ , and perhaps is a pathway for  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$  as well.<sup>31,32</sup> Absorbed Cu moves through the enterocytes bound



**Fig. 4** Plot of urinary Ni versus the inhalable water-soluble Ni subfraction for the combined group of electrorefinery and smelter departments workers.

to chaperone proteins and is exported to the portal circulation for delivery to the liver by the translocator protein ATP7A.<sup>30</sup> The uptake and intracellular shuttling of Cu in the hepatocytes is similar to that described for the absorptive cells, with a different but similar translocator (ATP7B) involved in transferring Cu to cuproenzymes (including ceruloplasmin) and its excretion from the liver into bile.<sup>33,34</sup> Secretion into bile takes place when hepatocyte concentrations of Cu are in excess. Mutations in ATP7A results in the congenital Menkes’ disease (copper deficiency), while inborn errors in ATP7B result in Wilson’s disease (Cu toxicity).<sup>30,34</sup>

For Cu, the lack of response of urinary excretion to the observed air Cu concentrations (Fig. 3) might have been expected, based on the tightly regulated Cu balance summarized above. Cu absorbed through the lung tissue perhaps will also participate in the homeostatic feedback, because the DMT-1 transporter appears to be operative in lung tissue<sup>32,35</sup> and since airways clearance may be expected to result in the transfer of deposited Cu to the gastrointestinal tract.<sup>36</sup>

For Ni, a relatively large proportion of the air samples were at the low end of the observed concentration range and a few at high concentrations (Fig. 4), thereby introducing potential biases which likely obscured any association in this instance. This same sampling issue also applies to Co (data not shown). Further, for all 3 metals, we are dealing with relatively low urine metal concentrations as well. Because of this, it is likely that any expected relationship between the water-soluble aerosol subfraction and urinary excretion is also influenced by competing factors, such as variation in particle mass and size distribution, as well as dietary factors that influence Cu bioavailability.<sup>37</sup> In part one, for four electrorefinery workers conducting similar work, the thoracic fraction for Ni varied between 13 and 62% of the inhalable fraction, while for pyrometallurgical workers the range was 23 to 62%. Similarly, the respirable fraction varied between 3 and 34% for the electrorefinery group and 10–43% for the smelter workers. Consequently, differences in respiratory tract deposition and clearance patterns (including swallowing), might have occurred, as well as individual factors such as hygiene while at

work and diet (including iron status).<sup>30,37</sup> Although Ni<sup>2+</sup> and Cu (as Cu<sup>+</sup> rather than Cu<sup>2+</sup>)<sup>31–33</sup> potentially share the DMT-1 transporter, the relatively low Ni exposures experienced by the Cu-refinery workers argue against invoking uptake competition.

## References

- 1 Y. Thomassen, E. Nieboer, N. Romanova, A. Nikonov, S. Hetland, E. P. VanSpronsen, J. Ø. Odland and V. Chaschin, *J. Environ. Monit.*, 2004, **6**, 985.
- 2 J. Ø. Odland, V. P. Tchatchchine, V. Bykov, P. E. Fiskebeck, E. Lund, Y. Thomassen and E. Nieboer, *Int. Arch. Occup. Environ. Health*, 1999, **72**, 151.
- 3 A. Askergren and M. Mellgren, *Scand. J. Work Environ. Health*, 1975, **1**, 45.
- 4 V. N. Finelli, P. Boscolo, E. Salimei, A. Messineo and G. Carelli, *Proceedings of the 4th International Conference on Heavy Metals in the Environment*, Edinburgh, UK, 1981, pp. 475–478.
- 5 I. Suci, L. Prodan, V. Lazar, E. Ilea, A. Cocirla, L. Olicini, A. Paduraru, O. Zagreanu, P. Lengyel, L. Gyrfi and D. Andru, *Med. Lav.*, 1981, **3**, 1900.
- 6 National Institute of Occupational Safety and Health, *Health hazard evaluation report No. HHE-78-132-818*, Copper Division Southwire Company, Inc., Carrollton, Springfield, Virginia, US Department of Commerce, National Technical Information Service (PB82-188632), 1981.
- 7 Institute of Medicine, *Dietary reference intake for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc*, National Academy Press, Washington, USA, 2002, <http://www.nap.edu/catalog/10026.html>.
- 8 J. R. Turnlund, K. C. Scott, G. L. Peiffer, A. M. Jang, W. R. Keyes, C. L. Keen and T. M. Sakamashi, *Am. J. Clin. Nutr.*, 1997, **65**, 72.
- 9 D. B. Milne, *Am. J. Clin. Nutr.*, 1998, **67**, 1041S.
- 10 W. B. Pratt, J. L. Omdahl and J. R. J. Sorenson, *Am. J. Clin. Nutr.*, 1985, **42**, 681.
- 11 F. Pizarro, M. Olivares, R. Uauy, P. Contreras, A. Rebelo and V. Gidi, *Environ. Health Perspect.*, 1999, **107**, 117.
- 12 S. Bügel, A. Harper, E. Rock, J. M. O'Connor, M. P. Bonham and J. J. Strain, *Br. J. Nutr.*, 2005, **94**, 231.
- 13 J. R. Turnlund, *Am. J. Clin. Nutr.*, 1998, **67**, 960S.
- 14 J. R. Turnlund, W. R. Keyes, G. L. Peiffer and K. C. Scott, *Am. J. Clin. Nutr.*, 1998, **67**, 1219.
- 15 Agency for Toxic Substances and Diseases Registry, *Toxicological Profile for Copper*, US Department of Health and Human Services, 2004.
- 16 Agency for Toxic Substances and Diseases Registry, *Toxicological Profile for Cobalt*, US Department of Health and Human Services, 2004.
- 17 Agency for Toxic Substances and Diseases Registry, *Toxicological Profile for Nickel*, US Department of Health and Human Services, 2005.
- 18 J. Borak, H. Cohen and T. A. Hethmon, *AIHAJ*, 2000, **61**, 832.
- 19 Y. Thomassen, E. Nieboer, D. Ellingsen, S. Hetland, T. Norseth, J. Ø. Odland, N. Romanova, S. Chernova and V. P. Tchatchchine, *J. Environ. Monit.*, 1999, **1**, 15.
- 20 D. J. Baern, M. M. McLean and O. R. Flores, *Clin. Chem.*, 1977, **23**, 1172.
- 21 E. Nieboer, W. E. Sanford and B. C. Stace, in *Nickel and Human Health. Current Perspectives. Advances in Environmental Science and Technology*, ed. E. Nieboer and J. O. Nriague, John Wiley, New York, USA, 1992, vol. 25, pp. 49–68.
- 22 B. Seifert, K. Becker, D. Helm, C. Krause, C. Schultz and M. Seiwert, *J. Exp. Anal. Environ. Epidemiol.*, 2000, **10**, 552.
- 23 T. Smith-Sivertsen, V. Tchatchchine, E. Lund, V. Bykov, Y. Thomassen and T. Norseth, *Environ. Health Perspect.*, 1998, **106**(8), 503.
- 24 J. R. Turnlund, C. L. Keen and R. G. Smith, *Am. J. Clin. Nutr.*, 1990, **51**, 658.
- 25 A. L. Rodgers, L. J. Barbour, B. M. Pougnet, C. J. Lombard and R. L. Ryall, *J. Trace Elem. Med. Biol.*, 1995, **9**, 150.
- 26 P. Hoet, J.-P. Buchet, L. Decerf, B. Lavallée, V. Haufroid and D. Lison, *Clin. Chem.*, 2006, **52**(1), 88.
- 27 M. A. Werner, J. H. Vincent, Y. Thomassen, S. Hetland and S. Berge, *Occup. Hyg.*, 1999, **5**, 93.
- 28 M. C. Linder and M. Hazegh-Azam, *Am. J. Clin. Nutr.*, 1996, **63**, 797S.
- 29 M. C. Linder, L. Wooten, P. Cerveza, S. Cotton, R. Shulze and N. Lomeli, *Am. J. Clin. Nutr.*, 1998, **67**, 965S.
- 30 P. Sharp, *Proc. Nutr. Soc.*, 2004, **63**, 563.
- 31 B. Mackenzie and M. D. Garrick, *Am. J. Physiol. Gastr. L.*, 2005, **289**, G981.
- 32 M. D. Garrick, K. G. Dolan, C. Horbinski, A. J. Ghio, D. Higgins, M. Porubcin, E. G. Moore, L. N. Hainsworth, J. N. Umbreit, M. E. Conrad, L. Feng, A. Lis, J. A. Roth, S. Singleton and L. M. Garrick, *Biometals*, 2003, **16**, 41.
- 33 M. Arredondo and M. T. Nunez, *Mol. Aspects Med.*, 2005, **26**, 313.
- 34 P. de Bie, B. van de Sluis, L. Klomp and C. Wijmenga, *J. Hered.*, 2005, **96**(7), 803.
- 35 J. L. Turi, F. Yang, M. D. Garrick, C. A. Piantadosi and A. J. Ghio, *Free Radical Biol. Med.*, 2004, **36**(7), 850.
- 36 E. Nieboer, G. G. Fletcher and Y. Thomassen, *J. Environ. Monit.*, 1999, **1**, 1.
- 37 B. Lönnerdal and B. Sandström, in *Handbook of Metal–Ligand Interactions in Biological Fluids: Bioinorganic Medicine*, ed. G. Berthoin, Marcel Dekker, New York, 1995, vol. 1, pp. 331–337.