

Determination of germanium in urine and its usefulness for biomonitoring of inhalation exposure to inorganic germanium in the occupational setting†

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The present study aimed to assess whether urinary germanium concentration can be used as a biomarker of inhalation exposure to airborne dust from metallic germanium (Ge) or GeO₂ in the occupational setting. A novel hydride generation-based method coupled with flow-injection graphite furnace atomic absorption spectrometry (HG/FI-GFAAS) was developed for the determination of urinary germanium. It was found that urinary germanium concentration could be reliably determined by a standard addition method after thorough digestion of the urine and careful pH adjustment of the digest. The limit of detection (LOD) in urine for the HG/FI-GFAAS method was 0.25 µg Ge L⁻¹. In Belgian control male subjects, the urinary germanium concentration was below this LOD. In 75 workers currently exposed to inorganic germanium compounds, respirable and inhalable concentrations of germanium in the aerosols were measured on Monday and Friday at the job sites using personal air samplers. Spot-urine samples were collected on the same days before and after the work shift. The germanium concentrations of respirable dust correlated very well with those of inhalable dust and represented 20% of the inhalable fraction. Workers exposed to metallic Ge dust were on average ten times less exposed to germanium than those whose exposure involved GeO₂ (3.4 versus 33.8 µg Ge m⁻³). This difference was reflected in the urinary germanium concentrations (3.4 versus 23.4 µg Ge g⁻¹ creatinine). Regression analysis showed that the concentration of germanium in the inhalable fraction explained 42% of the post-shift urinary germanium concentration either on Monday or on Friday, whereas in a subgroup of 52 workers mainly exposed to metallic germanium dust 57% ($r=0.76$) of the Monday post-shift urinary germanium was explained. Urinary elimination kinetics were studied in seven workers exposed to airborne dust of either metallic Ge or GeO₂. The urinary elimination rate of germanium was characterised by half-times ranging from 8.2 to 18.1 h (on average 12 h 46 min). The present study did not allow discrimination between the germanium species to which the workers were exposed, but it showed fast urinary elimination kinetics for inhalation exposure to dust of metallic Ge and GeO₂. It pointed out that urine samples taken at the end of the work shift can be used for biological monitoring of inorganic germanium exposure in the occupational setting.

Aim of investigation

Germanium (Ge) occurs widely dispersed in the Earth's crust with an abundance varying from 1.5 to 7 ppm.¹ It is invariably found in living organisms, but there is little or no circumstantial evidence for the essentiality of germanium as trace element for plants, animals, or humans.^{2,3} In the general population, food is the major source of intake of germanium. The daily dietary intake of germanium in adults has been found to be 0.37 mg in the UK,⁴ whereas an amount of 1.5 mg has been calculated for the USA.⁵ Foodstuffs such as garlic, ginseng, oats, seafood, and tomato juice may contain substantial amounts of this metal which is very well absorbed by the gastrointestinal tract.² In humans, it has been reported that after oral intake of a single germanate dose the absorption rate was 96% in 8 h and the elimination half-time of germanium was 1.5 days.^{1,6} For a reference man, balance studies indicated excretion rates of germanium that amounted to 93.3% for urine and 6.7% for feces.¹ In the 1980s, germanium-based nutritional supplements became very popular as "health-producing

elixirs" in Japan⁷ and also in Europe.⁸ However, the long-term oral intake of these over-the-counter preparations (containing GeO₂, carboxyethyl germanium sesquioxide, or germanium lactate-citrate) may represent a serious health hazard as documented by many case reports on ingestion of large doses, showing not only severely impaired kidney function (at least nine fatal cases of renal failure) but also other health effects, such as anaemia, myopathy, and peripheral neuropathy.⁷⁻¹⁰

Unlike the well-documented toxicological database on oral intake of germanium compounds, much less is known about the inhalation toxicity of inorganic germanium. We are not aware of any controlled study in humans allowing the assessment of the pulmonary absorption and urinary excretion rates of inorganic germanium compounds. There is one old investigation in rodents exposed to respirable particulate aerosols generated from neutron-activated ⁷¹Ge metallic dust or ⁷¹GeO₂ powder (mean diameter: 1.7 and 0.45 µm, respectively) that showed a relatively fast disappearance of radio-germanium from the lungs (for ⁷¹Ge, 52% at 1st and 82% at 7th day; for ⁷¹GeO₂, 79% at 1st and nearly 100% at 4th day) and a rapid urinary elimination.^{11,12}

Germanium is a precious by-product of non-ferrous

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metallurgy and is of economic and strategic importance.⁶ The global production of the most important inorganic germanium compounds (*viz.* metallic Ge, GeCl₄, and GeO₂) varies between 30 and 80 metric tonnes per year.^{1,6} Metallic Ge is used in the manufacture of lenses and windows for infra-red optics, semiconductor electronics, and extremely pure Ge single-crystals for high-energy radiation photodetectors. High-purity GeCl₄ is an important compound in the fabrication of optical fibres for telecommunication. GeO₂ is used in the poly(ethylene terephthalate) production as a catalyst, in the synthesis of organogermanium compounds, and in the manufacture of phosphorescent germanate crystals or crystals useful for scintillation detection.⁶ Chronic occupational exposure to germanium mainly occurs through inhalation of airborne germanium-containing dust generated from the production and utilisation of metallic Ge or GeO₂ powder. Stokinger¹³ concluded from animal experiments that these two germanium compounds are of comparatively low toxicity by all routes of administration tested including inhalation which led to the consideration of them as hardly an industrial health hazard for humans.^{6,14,15} Recent findings, however, suggest that the germanium risk at the workplace may need adequate surveillance.¹⁶

In the industrial setting, the exposure intensity to a toxic compound is usually assessed by monitoring its atmospheric concentration through personal and/or workroom air sampling at the job sites (external dose). This approach has limitations as it does not take into account personal differences (*e.g.*, age, gender, height, weight, physical aptitude, physiological and nutritional status, duration of exposure), the possibility of other routes of exposure (*viz.*, oral or dermal), or inter-individual differences as to exposure, absorption, distribution, biotransformation, and excretion. Assessment of the absorbed quantity of the toxic compound (internal dose) through a biological method takes into account most of these individual parameters.¹⁷

The present study in workers exposed to inorganic germanium compounds deals with both exposure assessments. However, to assess the internal dose we needed to develop a novel analytical method allowing a reliable determination of germanium in urine which posed a real challenge as to sensitivity and accuracy requirements because the metal is only present in trace amounts and the biomatrix of urine is variable and complex. Spectrophotometric methods based on the formation of methylene blue–molybdogermanate¹⁸ or Ge(IV) phenylfluorone^{19,20} complexes had limits of detection (LODs) of 7 and 48 µg Ge L⁻¹, respectively, too high for adequate determinations in human urine. Flame atomic absorption spectrometry (AAS) showed poor sensitivity because Ge tends to form stable oxide species in the flame, and graphite furnace AAS (GFAAS) methods often suffered from losses of volatile germanium compounds (GeO, GeCl₄) formed during the incineration stage.^{21–23} CCl₄-based extraction of acid digested biomaterials combined with the use of Co(NO₃)₂ as matrix modifier in GFAAS analysis has been shown to suffer from losses of germanium during the extraction step and the LOD (4.5 µg Ge L⁻¹) was still insufficient for the measurement of Ge in urine.²⁴ Hydride generation (HG) AAS methods involving GeH₄ gas formation in combination with a graphite furnace showed detection limits from 1.4 ng Ge L⁻¹ for 100 mL water samples²⁵ to 100 µg Ge L⁻¹ for 20 µL aqueous solutions.²⁶ The breakthrough for small volume samples came when the electrographite tube was used as both the hydride trapping cell and the atomisation cell in combination with a flow-injection (FI) system.^{27,28} A significant improvement of this concept has been introduced by Shuttler *et al.*²⁹ through the application of a manual injection of 0.1 mL of a Pd–Ir-based trapping reagent into the electrographite tube followed by a specific thermal treatment of the tube.

For the present study, we took advantage of the analytical

findings of Shuttler *et al.*²⁹ and developed a novel HG-based/FI-GFAAS method (HG/FI-GFAAS) in order to reach a sufficiently low detection limit for the determination of germanium in urine. Personal air samples and urine samples were collected in germanium-exposed workers to assess the validity of urinary germanium as biomarker of occupational exposure to airborne inorganic germanium. We also made a limited attempt to throw some light on the urinary elimination kinetics of inorganic germanium in humans.

Site description⁶

Dry germanium concentrate from zinc mines is treated by Union Minière-Olen, Belgium, and involves leaching, solvent extraction, and precipitation/concentration by hydrolysis. The following step comprises the transformation of germanium concentrate (mostly GeO₂) or Ge metal scrap to germanium which relies on the volatility of GeCl₄ as the separation–purification step. Purified GeCl₄ is hydrolysed with high-purity water and after filtration and thorough washing, the GeO₂ is dried. Pure GeCl₄ and GeO₂ are made commercially available. Elemental Ge (sponge) is produced by hydrogen reduction of pure GeO₂ in electrically heated furnaces at 650 °C. Subsequently, the Ge sponge undergoes zone-melting refining and ultra-purification at temperatures above the melting point [937 °C] generating polycrystalline or semiconductor-grade Ge. Extreme purity levels are only obtained by crystal pulling to yield dislocation-free single crystals. Crystalline Ge is ground, sliced, and milled with great care to obtain the desired geometrical shapes, operations which generate Ge dust and scrap fines.

Study design

In the present study, we assessed the relation between external (air) and internal (urine) germanium exposure in a group of 75 workers from the Belgian plant of whom 50 were exposed to metallic Ge dust, six to GeO₂ and Ge metal dust, six to GeO₂ and GeCl₄ dust, and 13 had variable exposures. Personal air samples were taken on Monday and Friday during a whole shift (usually from 6.00 to 14.00 h) of a typical working week and also pre- and post-shift spot-urine samples were collected from each worker on the same day of the air sampling. Respiratory masks were not worn.

In seven of those workers, we investigated the elimination rate of germanium in urine on another occasion when they finished their work on Friday afternoon and had the weekend off. Spot-urine samples were taken on Friday before and after the shift. The workers were then instructed to collect at home spot-urine samples in the morning, afternoon, and evening of Saturday and Sunday. The last urine sample was taken just before they resumed their work on Monday morning.

Experimental procedures

Chemicals and reagents

All chemicals used were of *pro analysi* grade: *viz.*, H₂SO₄ (95%, *d*=1.84), HNO₃ (65%, *d*=1.40), H₃PO₄ (85%, *d*=1.71), NH₃ (25%, *d*=0.91), ethanol (96% m/v), *m*-cresol purple (*m*-cresolsulfophthalein), and chlorophenol red (dichlorophenol-sulfophthalein), IrCl₃, and Mg(NO₃)₂ (Merck, Darmstadt, Germany); NaOH (solid), H₂O₂ (30% m/v), and KH₂PO₄ (Fluka Chemie AG, Buchs, Switzerland); NaBH₄ (solid) and germanium standard solution (1 g Ge L⁻¹) for AAS (Sigma-Aldrich); argon gas 99.999% (Air Liquide, Belgium). Dilutions were made using de-ionized water (Nanopure, Sybron/Barnstead, Boston).

Potassium phosphate buffer 0.4 mol L⁻¹, pH 6, was prepared by dissolving 108.9 g KH₂PO₄ in 1 L water, adding

17.5 mL NaOH 10 mol L⁻¹ (40 g NaOH dL⁻¹), and completing the volume to 2 L with water. The pH was adjusted to 6 by adding NaOH 10 mol L⁻¹ or H₃PO₄ (85%). Potassium phosphate buffer 0.2 mol L⁻¹, pH 6, was obtained by dilution of the former. A pH-indicator mixture was prepared by dissolving 15 mg *m*-Cresol Purple and 25 mg Chlorophenol Red in 25 mL ethanol 20%. NaBH₄ solutions (0.4% m/v) were freshly prepared each day by adding 2 g of solid NaBH₄ to 50 mL of water containing 100 mg NaOH, then the volume was brought to 500 mL with water. This solution was kept in an ice bath during the analysis. The coating/trapping reagent was prepared by mixing 9 mL of 1 mg Ir mL⁻¹ solution with 1 mL of 10 mg Mg mL⁻¹ solution, yielding an iridium–magnesium solution of 0.09 and 0.1% m/v for iridium and magnesium, respectively. Germanium calibration solutions were prepared by dilution of the 1 g Ge L⁻¹ standard solution with potassium phosphate buffer 0.2 mol L⁻¹, pH 6, to obtain a series of solutions of which the germanium concentration varied from 0 (blank) to 50 µg Ge L⁻¹ for HG/FI-GFAAS and from 0 (blank) to 250 mg Ge L⁻¹ for flame AAS measurements. Potassium phosphate buffer 0.2 mol L⁻¹, pH 6, was used as blank. The solutions were kept at 4 °C and used for up to 6 months.

Pre-treatment of graphite furnace atomiser

For the HG/FI-GFAAS analysis of germanium, a Perkin-Elmer Zeeman 4100ZL atomic absorption spectrometer was used which was equipped with a Perkin-Elmer transverse heated graphite atomiser (THGA) in which a pyrolytic graphite coated electrographite tube (part no. B3000655) of transverse geometry was mounted. Each day a new tube with L'vov platform (referred to as the THGA tube) was used. Before starting the analyses of germanium, two specific pre-treatments were needed for the THGA tube. First came a thermal treatment comprising five consecutive firing cycles under continuous argon flow (250 mL min⁻¹) each interrupted by a cold step (20 °C, ramp 1 s, hold 20 s), *viz.* 2000 °C (ramp 60 s, hold 5 s), 2200 °C (ramp 10 s, hold 10 s), 2300 °C (ramp 10 s, hold 10 s), 2400 °C (ramp 10 s, hold 5 s), and 2500 °C (ramp 1 s, hold 5 s). Subsequently, 40 µL of the Ir–Mg coating/trapping reagent was injected manually with an Eppendorf pipette onto the L'vov platform and then a thermal coating cycle was applied under continuous argon flow (250 mL min⁻¹), *viz.* 100 °C (ramp 2 s, hold 40 s), 130 °C (ramp 20 s, hold 60 s), 1100 °C (ramp 20 s, hold 60 s), 2300 °C (ramp 0 s, hold 5 s), and 2400 °C (ramp 1 s, hold 1 s). The latter procedure was repeated twice to obtain a sufficiently stable Ir–Mg coating to allow a full day of continuous measurements.

Sample clean-up and HG/FI-GFAAS method for Ge determination in urine

The novel method comprised four consecutive stages, *viz.* acid digestion of urine, adjustment of pH, generation of GeH₄ gas, trapping of the GeH₄ gas and electrothermal atomisation. We describe here the optimised operational procedure as it emerged from the chemical, analytical, and instrumental method development of which the details are to be dealt with in a separate paper.

(i) *Wet digestion.* Typical aliquots of 15 mL urine were digested in open pyrex tubes each containing a few glass beads and were placed in a Tecator Digestion System 40 (Digester 1016 and Autostep 1012 Controller). Before use, glass and pyrex ware was soaked in NaOH 1 mol L⁻¹ and rinsed with Nanopure water. The digestion comprised three steps: 1st step, add 5 mL HNO₃ 14 mol L⁻¹ to the urine and warm up to 75 °C (ramp 1 h, hold 30 min); 2nd step, cool down, add 2.5 mL HNO₃ 14 mol L⁻¹ and 5 mL H₂O₂ 30%, and heat successively to 100 °C (ramp 6 h, hold 30 min) and 120 °C (ramp 3 h, hold

15 h) to reduce the volume to about 1 mL (avoid dryness); 3rd step, cool down, add 2 mL Nanopure water and 2 mL H₂SO₄ 18 mol L⁻¹, mix well, add 5 mL H₂O₂ 30%, and heat successively to 50 °C (ramp 45 min, hold 2 h), 80 °C (ramp 6 h, hold 1 h), 150 °C (ramp 4 h, hold 2 h), and 180 °C (ramp 1 h, hold 2 h) to evaporate the remaining nitric acid (avoid dryness). If the resulting digests (about 2 mL) were not colourless, 0.25 mL of H₂O₂ 30% was added and the mixture heated at 70 °C until disappearance of the brownish colour.

(ii) *pH adjustment.* The H₂SO₄-based digests were diluted each with 15 mL potassium phosphate buffer 0.4 mol L⁻¹, pH 6, and then 0.2 mL of the pH-indicator mixture was added to allow visual pH monitoring upon addition of NH₃ 13.5 mol L⁻¹ in aliquots of 0.25 mL until the colour shifted from red to yellow (pH zone 1.2–2.8); subsequently a drop of NH₃ 13.5 mol L⁻¹ at the time was added until the yellow colour turned persistently to mauve (pH zone 4.8–6.4). Eventually, the pH was adjusted to a value between 5.5 and 6.0 using diluted H₂SO₄ or NH₃ solutions while controlling with a Gilson pH meter. The mixtures were further diluted with Nanopure water to a final volume of 30 mL and stored at 4 °C in capped polystyrene tubes (Nunc, Life Technologies, Merelbeke, Belgium).

(iii) *Generation of GeH₄.* Gaseous GeH₄ was generated using a set-up comprising a Perkin-Elmer autosampler AS-90 linked to a hydride generation/flow-injection analysis system (Perkin-Elmer HG/FIAS-400) consisting of a multi-pump/channel/valve-system programmed to carry out the following steps on-line: sampling of a fixed volume of analyte solution (sample loop: 0.5 mL) and mixing with the carrier stream (potassium phosphate buffer 0.2 mol L⁻¹, pH 6), then transport to the reaction cell for mixing the sample with NaBH₄ 0.4% solution. The *in situ* generated GeH₄ gas was separated by passing the reaction mixture through a bed of small glass beads functioning as a gas/liquid separator with argon as sweeping gas (flow 20–30 mL min⁻¹).

(iv) *Trapping of GeH₄ and atomisation.* GeH₄ was swept away by the argon flow and led *via* Teflon tubing to a Perkin-Elmer autosampler AS-70 equipped with a quartz capillary at the injection tip. The GeH₄ was injected and trapped into the Ir–Mg coated THGA tube which was heated at 400 °C during the injection sequence. The generation, injection, and trapping of GeH₄ occurred on-line in a synchronised timed sequence lasting about 80 s. It is believed that during this step GeH₄ adsorbs onto the surface of the coated graphite which in turn catalyses its decomposition in Ge and H₂. The actual electrothermal atomisation programme comprised three steps: 1st step, purge of the oven with an argon flow of 250 mL min⁻¹ at 400 °C (ramp 1 s, hold 5 s); 2nd step, atomisation of the trapped Ge at 2400 °C (stop argon flow, ramp 0 s, hold 6 s); 3rd step, cleaning of the oven at 2400 °C (argon flow 250 mL min⁻¹, ramp 1 s, hold 5 s). The atomic absorption signal of Ge was measured at 265.1 nm (Perkin-Elmer 'System 2' electrodeless discharge lamp) with a slit setting of 0.2 nm and integrated over the 6 s period of the actual Ge atomisation lasted (rollover as from 1.8 units of time-integrated absorbance).

An experimental gas/liquid separator in glass and the default spectrometer conditions automatically recalled by the software were obtained from Perkin-Elmer, Überlingen, Germany. The complete system was controlled by a GEM (Digital Research) based PEAALABS software running on a PC equipped with an IEEE-488 interface allowing communication with the Zeeman 4100ZL atomic absorption spectrometer, the autosamplers AS-90 and AS-70, and the HG/FIAS-400. Acquisition and treatment of data were carried out with the same PC. The germanium concentration in urine was expressed either in µg Ge L⁻¹ or in µg Ge g⁻¹ creatinine to normalise for urine dilution.

Personal air sampling and determination of Ge in air samples

Ambient air sampling was carried out during the whole workshift (about 8 h) with CIP-10 battery-operated personal dust samplers (MSA, Saint Ouen-l'Aumône, France) at a flow rate of 10 L min^{-1} . This type of air sampler allows adequate sampling of the inhalable dust fraction as defined by the ISO-CEN-ACGIH convention.^{30,31} Aerosol particulate of metallic Ge and/or GeO_2 was collected at three levels on polyurethane foam filters of which the last one collects the respirable dust particles only. Each filter was separately dissolved in HNO_3 14 mol L^{-1} and heated at 120°C to oxidise most of the organic material. Then H_2O_2 was added to complete the oxidation and transformation of germanium in the Ge(IV) oxidation state.^{32,33} It should be pointed out that metallic germanium in the form of dust or powder readily reacts with concentrated nitric acid.³⁴ The pale-yellow residues were brought into solution in 20 mL 0.1 mol L^{-1} NaOH. Solutions containing more than 1.5 mg Ge L^{-1} were analysed with flame AAS ($\text{N}_2\text{O}/\text{C}_2\text{H}_2$) using a Varian Spectra AA-10, while the above described HG/FI-GFAAS method was used for the other solutions after a 100 or 200-fold dilution with potassium phosphate buffer, 0.2 mol L^{-1} , pH 6. The latter method allowed the measurement of airborne concentrations as low as 10 ng Ge m^{-3} .¹⁶

Results and discussion

Analytical characteristics of the HG/FI-GFAAS method and validation of the procedure

The method for the determination of germanium in urine as described above has been tested for the classic analytical parameters. Exploratory assays were run to fix the settings of the multi-pump/channel/valve-system of the FIAS-400 in order to obtain a linear response of the atomic absorption signal for a sample-loop of 0.5 mL . A calibration line from 0 to $50 \text{ } \mu\text{g Ge L}^{-1}$ in potassium phosphate buffer, 0.2 mol L^{-1} , pH 6, showed a linear relationship for germanium concentrations up to a peak area (absorbance \times time) of 0.700 corresponding to about $20 \text{ } \mu\text{g Ge L}^{-1}$. The LOD of the HG/FI-GFAAS method was determined as three times the standard deviation above potassium phosphate buffer blanks ($n=15$) and amounted to $0.22 \text{ } \mu\text{g Ge L}^{-1}$ or 110 pg Ge in absolute quantity. The AAS signal of Ge was found to be quenched by the digestion matrix and to check this effect urine samples from eight different control persons were digested as described above and spiked with $5 \text{ } \mu\text{g Ge L}^{-1}$ just before the pH adjustment. Three times 5 mL of each digested sample were measured using a standard additions method (0, 12.5 or 25 ng of germanium added). Compared to a calibration line in potassium phosphate buffer, 0.2 mol L^{-1} , pH 6, the recovery of added Ge varied from 81 to 99% (mean 89.4%, RSD 7%). To allow for the variable influence of the digestion matrix, we concluded that for reliable results urinary germanium must be determined using the method of standard additions, that the regression line must be linear and the correlation coefficient at least 0.999. In these conditions the LOD for germanium in urine was set at $0.25 \text{ } \mu\text{g Ge L}^{-1}$. The analytical repeatability was tested using two series of 10 control urine aliquots spiked with either 8 or $100 \text{ } \mu\text{g Ge L}^{-1}$ and which underwent the whole clean-up procedure. If Ge concentrations were beyond the linear AAS requirements, an appropriate dilution of the digested urine with potassium phosphate buffer, 0.2 mol L^{-1} , pH 6, was needed before the standard addition method could be applied. From each series five samples were analysed on the same day and five on different days. The RSDs were 1.4 and 3.5% (within day) and 2.2 and 3.9% (between days) for the low and high spikes, respectively. Because certified reference material for urinary germanium was not commercially available, the accuracy of the whole procedure was tested with home-made spiked urine

aliquots prepared from a control urine that was spiked at three different concentration levels, viz. $0.2 \text{ } \mu\text{g Ge L}^{-1}$ ($n=8$), $8 \text{ } \mu\text{g Ge L}^{-1}$ ($n=15$), and $100 \text{ } \mu\text{g Ge L}^{-1}$ ($n=8$). After appropriate dilution of the digested spiked urine samples, the standard addition method was applied to determine germanium. Digested non-spiked urine served as blank. An accuracy of 106% (RSD 12.5%), 98.7% (RSD 2.1%), and 96% (RSD 3.8%) was found for the 0.2, 8, and $100 \text{ } \mu\text{g Ge L}^{-1}$ spikes, respectively. The higher variability for the $0.2 \text{ } \mu\text{g Ge L}^{-1}$ spike is most likely due to the fact that the concentrations were close to the LOD of the present method.

In typical routine conditions, batches of duplicate aliquots of 19 urine specimen were prepared and two blanks (15 mL water). The wet digestion step took about two days to obtain the final H_2SO_4 -based colourless mineralised urine, because the digestion temperature must be increased very slowly to limit a too violent oxidation reaction of H_2O_2 and to enable in H_2SO_4 the H_2O_2 -mediated production of monoperoxo-sulfuric acid which is a very strong oxidant. These precautions would also help to avoid possible loss of volatile germanium compounds, e.g. GeO , and similarly, we do not know whether biotransformation of inorganic germanium would occur in animals or humans that would lead to the excretion of methylated germanium derivatives in urine.¹⁵ As the whole digestion procedure is programmed and auto-controlled, it takes only a minimum of a technician's time to add the different reagents and to supervise the digestion. The pH adjustment is a crucial step and needs time, whereas the HG/FI-GFAAS method is fully automated taking about 4 min per run of germanium measurement. The Ir-Mg coating as applied in the present procedure provides sufficient stability for the completion of 90 to 120 trapping/atomisation cycles that roughly corresponds to 6–8 h of work. This is at variance with Shuttler *et al.*²⁹ who obtained a coating (Pd-Ir) stability of 300 cycles for the determination of selenium, however, it should be pointed out that the atomisation temperature for Se is only 2050°C . Preliminary tests have shown that at an atomisation temperature of 2100°C no atomic absorption signal for Ge is produced and that in terms of number of complete trapping/atomisation cycles the lower performance of the present HG/FI-GFAAS method is most likely due to the much higher atomisation temperature needed for Ge.

Normal urinary germanium levels in adults

In the present study, the mean of duplicate measurements was calculated and the whole procedure was repeated on urine samples whose duplicate analyses differed by more than 10%. In subjects with no occupational exposure to germanium, the urinary germanium concentration is $<1 \text{ } \mu\text{g Ge g}^{-1}$ creatinine.³⁵ Despite an adequate sensitivity of the present procedure (LOD $0.25 \text{ } \mu\text{g Ge L}^{-1}$ of urine), germanium was not detectable in urine specimen collected in more than 70 Belgian adult male control subjects. The figures quoted in the literature for the urinary concentration of germanium vary considerably and should be taken with caution as regional differences may exist and/or inadequate analytical techniques may have been used. For instance, the urinary excretion of germanium in normal human adults has been reported to range as from 0.56 to 3.0 mg per day,¹⁴ a mean concentration of $1.26 \text{ mg Ge L}^{-1}$ of urine was found in four US citizens,³⁶ whereas germanium could not be detected (LOD $8 \text{ } \mu\text{g Ge L}^{-1}$) in the urine of Japanese control subjects with renal failure.⁷

Relation between air and urine germanium in the occupational setting

The distributions of the concentrations of atmospheric and urinary germanium were positively skewed. They were normal-

Table 1 Atmospheric and urinary concentrations of germanium in the workforce exposed to inorganic germanium compounds

	Monday		Friday	
	GM (GSD) ^a	Min-max	GM (GSD) ^a	Min-max
Ge in air/ $\mu\text{g Ge m}^{-3}$				
Respirable ($n=75$)	1.07 (3.21)	0.03–40.7	0.92 (3.98)	0.02–37.2
Inhalable ($n=75$)	5.11 (7.63)	0.07–343.9	5.11 (9.19)	0.03–292.5
Ge in urine/ $\mu\text{g Ge g}^{-1}$ creatinine				
Pre-shift ^b	1.36 (2.50)	0.23–20.2	3.16 (3.61) ^c	0.30–70.8
Post-shift ($n=75$)	4.22 (4.96) ^d	0.16–160.0	4.34 (5.86) ^e	0.12–194.6

^aGeometric mean and geometric standard deviation. ^bOn Monday 65 and on Friday 69 pre-shift urine samples had germanium concentrations above the LOD; all 75 post-shift urine samples, either on Monday or on Friday, had concentrations $>0.25 \mu\text{g Ge L}^{-1}$. ^cBonferroni multiple comparison test: $0.05 < p < 0.1$ vs. Monday pre-shift urine. ^dBonferroni multiple comparison test: $p < 0.01$ vs. Monday pre-shift urine. ^e $p < 0.001$ vs. Monday pre-shift urine.

used by logarithmic transformation. The geometric means of the atmospheric concentrations for respirable and inhalable germanium are shown in Table 1 together with the corresponding germanium concentrations in urine. On a group basis, the respirable and inhalable concentrations of germanium in the aerosols did not differ significantly between Monday and Friday, so that it can be assumed that the external germanium exposure was rather constant in time at the job sites. On average the respirable fraction amounted to 20% of the inhalable dust and both variables correlated very well on Monday [$R^2=0.916$; $\log\text{Ge}(\text{resp})=-0.6002+0.8887\log\text{Ge}(\text{inhalable})$] as well as on Friday [$R^2=0.844$; $\log\text{Ge}(\text{resp})=-0.6581+0.8744\log\text{Ge}(\text{inhalable})$]. As to the germanium concentration in the pre-shift urine samples, there were on Monday ten samples with germanium concentrations below the LOD and on Friday the number was six. All the post-shift urine samples, either on Monday or on Friday, had detectable germanium concentrations. The urine samples of Monday morning with detectable germanium had an average concentration of $1.36 \mu\text{g Ge g}^{-1}$ creatinine, which is substantially higher than in control subjects for whom the urinary germanium concentration was below the LOD. This indicated that after the weekend, during which there was no germanium exposure, the urinary germanium concentration in the majority of the workers did not drop to normal control values and that thus germanium inhaled during the week before is still eliminated *via* the urine. It should be pointed out that the germanium concentration in the pre-shift urine samples of Friday was 2.3 times higher than the corresponding samples of Monday suggesting that there may be a build-up of germanium over the course of the work week. The germanium concentrations in the post-shift urine samples of Monday and Friday did not differ significantly, they were about 35% higher than on Friday morning, and were significantly higher than in the pre-shift urine samples of Monday.

On a group basis, the type of job site seemed to influence to some extent the external exposure which was also reflected in the urinary germanium concentrations. In the fifty workers at job sites with only exposure to dust of metallic Ge, the geometric mean (GSD) of the germanium concentration for inhalable dust averaged on Friday $3.42 (1.28) \mu\text{g Ge m}^{-3}$ against $33.84 (5.88) \mu\text{g Ge m}^{-3}$ for the twelve workers whose exposure involved GeO_2 dust. The corresponding geometric mean values for the germanium concentrations in the post-shift urine samples of Friday were $3.37 (1.23)$ and $23.39 (4.30) \mu\text{g Ge g}^{-1}$ creatinine. Apart from the concentration factor, no distinct distribution pattern of the urinary germanium values was found that might relate to the chemical species of germanium.¹⁶ In the total worker population ($n=75$), the regression analysis showed for Monday and Friday that the post-shift urinary germanium concentration (in $\mu\text{g Ge g}^{-1}$ creatinine) was for 42% ($p < 0.001$) explained by the concentration of inhalable germanium (in $\mu\text{g Ge m}^{-3}$)

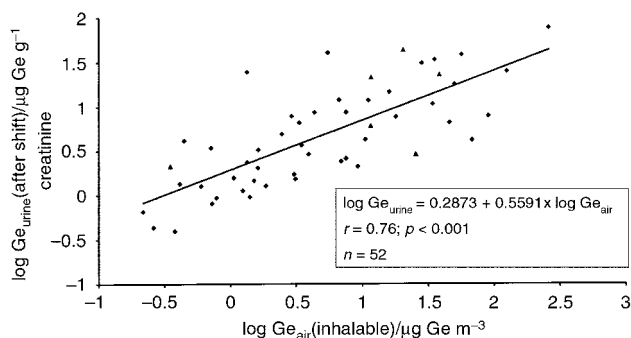


Fig. 1 Scatter plot and relationship between the concentration of germanium in post-shift urine samples as a function of the concentration of germanium in the inhalable aerosol fractions on Monday in workers exposed to metallic germanium dust (\circ) or metallic germanium and GeO_2 dust (\blacktriangle).

(Monday: $\log\text{Ge}_{\text{urine}}=0.2448+0.5367\log\text{Ge}_{\text{air}}$; Friday: $\log\text{Ge}_{\text{urine}}=0.2490+0.5473\log\text{Ge}_{\text{air}}$). We looked also more closely to the relationship between post-shift urinary germanium and atmospheric germanium measured on Monday and Friday in a subgroup ($n=56$) of workers with a clear-cut pattern of job site exposure. Therefore we combined the workers exposed to germanium metal dust ($n=50$) with the six workers whose exposure involved germanium metal dust and GeO_2 dust. For the regression analysis we deleted from this subgroup the data points having a urinary germanium concentration lower than $0.3 \mu\text{g Ge g}^{-1}$ creatinine (practical detection limit for urine). Three data points were deleted from the Monday data set and two from the Friday data set. One worker, the same on Monday and Friday, was also excluded from this analysis because of low urinary germanium in the presence of high air germanium. The regression analysis for the Friday data set ($n=53$) of post-shift Ge_{urine} (in $\mu\text{g Ge g}^{-1}$ creatinine) and inhalable Ge_{air} (in $\mu\text{g Ge m}^{-3}$) showed a correlation coefficient of 0.66 ($p < 0.001$) and the regression equation was $\log\text{Ge}_{\text{urine}}=0.3712+0.4540\log\text{Ge}_{\text{air}}$. The corresponding results for the data set of Monday ($n=52$) were $\log\text{Ge}_{\text{urine}}=0.2873+0.5591\log\text{Ge}_{\text{air}}$ ($r=0.76$; $p < 0.001$) (Fig. 1). The relationship was thus better for the Monday data set and may be due to the fact that after the weekend without exposure, the post-shift urinary germanium reflected better the germanium amount absorbed on the first workday of the work week, whereas the post-shift urine samples of Friday were most likely also influenced by the germanium exposure of the days before.

These results showed that post-shift germanium concentration in urine of workers occupationally exposed to inorganic germanium compounds unequivocally reflects current inhalation exposure. Hence, the measurement of urinary germanium may be useful for the biomonitoring of occupational exposure to inorganic germanium compounds.

Table 2 Time courses and half-lives of urinary germanium elimination (in $\mu\text{g Ge g}^{-1}$ creatinine) in seven workers followed up over the weekend when there was no exposure to inorganic germanium

	Exposure to metallic Ge dust			Exposure to GeO_2 dust			
	Cutting, polishing	Cleaning lenses	Etching, crystal pulling	Bagging	Grinding	Cleaning furnace/filter-distillation	
<i>Friday—</i>							
Pre-shift	0.8	1.5	138.1	50.6	284.3	597.0	229.7
Post-shift	11.9	5.6	203.1	76.7	1978.9	1570.2	2121.7
<i>Saturday—</i>							
Morning	—	3.9	24.8	25.2	—	278.2	402.6
Afternoon	5.0	3.5	32.8	25.0	460.4	103.4	419.6
Evening	3.0	2.8	7.5	—	—	65.6	125.3
<i>Sunday—</i>							
Morning	1.8	1.1	10.4	11.3	211.5	56.1	—
Afternoon	—	0.9	—	12.1	145.2	—	—
Evening	2.0	—	—	—	—	—	84.5
<i>Monday—</i>							
Pre-shift	0.6	—	5.6	2.6	12.4	—	31.0
Half-life/h ^a	18.13	15.13	12.39	14.40	8.60	8.20	12.54
r^b	0.971	0.987	0.873	0.973	0.957	0.967	0.978
p	<0.005	<0.001	<0.025	<0.005	<0.025	<0.01	<0.001

^aThe elimination kinetics were calculated using the germanium concentrations of the available urine samples collected as from Friday post-shift to Monday pre-shift. The exact time elapsed was calculated taking as $t=0$ the time when the post-shift urine sample was collected on Friday.
^bPearson correlation coefficient.

Urinary elimination kinetics of germanium

The time courses of germanium elimination in urine over the weekend when there was no occupational exposure to inorganic germanium are shown in Table 2 for seven workers who volunteered to continue to collect spot-urine samples at home during Saturday and Sunday. Three workers were exposed to dust of metallic germanium generated at job sites like cutting/polishing, cleaning lenses, or etching and crystal pulling. The four other workers were exposed to GeO_2 dust generated during their activities involving bagging of GeO_2 powder, grinding GeO_2 , or cleaning furnace/filter and distillation. Two workers were studied at the last mentioned job site which was known for rather high exposure. On Friday, each worker provided a pre- and post-shift urine sample. As shown in Table 2, the post-shift urine samples had invariably much higher germanium concentrations than the pre-shift samples reflecting the absorption during the past 8 h of exposure. This confirms what has been shown in Table 1 on a group basis and it also suggests that the germanium elimination *via* the kidney is rapidly influenced by the current exposure. For the study of the urinary elimination kinetics of germanium, we took the post-shift urinary germanium concentration of Friday as starting point ($t=0$) and measured the germanium concentrations in spot-urine samples collected by the workers at home. The exact time of each urine sample collection was provided by the workers. Two of them had collected four samples from Saturday morning to Monday morning and the other five collected five samples. This allowed the calculation of the elimination half-life of germanium in urine. The half-lives ranged from 8.20 to 18.13 h and amounted to 12.77 h on average (12 h 46 min). The Pearson correlation coefficients ranged from 0.873 to 0.987 with p -values ranging from <0.025 to <0.001. In this limited study, the half-times of urinary elimination did not allow discrimination between the inorganic germanium species. The present findings suggest fast elimination kinetics for urinary germanium following inhalation exposure. Orally absorbed germanium in the form of Ge-132 (carboxyethylgermanium sesquioxide) also showed rapid elimination kinetics as found after a single oral dose was given to 11 healthy college students.³⁷ The urinary excretion rate of germanium peaked at around 3 h and after 24 h the germanium concentration in urine returned to the value before dosing. Similarly, human patients treated with 25 to 75 mg kg^{-1} of Ge-132 had gastrointestinal absorption rates of 30% and very rapid urinary excretion.³⁸ These observations

are particularly relevant to the present study because roughly 80% of the inhalable fraction of the germanium-containing aerosol was found in the nonrespirable fraction. One may assume that a substantial part of it may deposit in the thoracic area and after ciliary clearance be swallowed and then absorbed by the gastrointestinal tract. The present limited study pointed out that the urine samples for biomonitoring of inhalation exposure to inorganic germanium should be collected immediately after the workers completed the work shift.

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