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The SAM-Krom biomonitoring study shows occupational exposure to hexavalent chromium and increased genotoxicity in Denmark

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ABSTRACT

Background: Hexavalent chromium (Cr(VI)) is a carcinogen. Exposure to Cr(VI) may occur in different industrial processes such as chrome plating and stainless steel welding. The aim of this study was to assess occupational exposure to Cr(VI) in Denmark.

Methods: This cross-sectional study included 28 workers and 8 apprentices with potential Cr(VI) exposure and 24 within company controls, all recruited from six companies and one vocational school. Use of occupational safety and health (OSH) risk prevention measures were assessed through triangulation of interviews, a questionnaire and systematic observations. Inhalable Cr(VI) and Cr-total were assessed by personal air exposure measurements on Cr(VI) exposed participants and stationary measurements. Cr concentrations were measured in urine and in red blood cells (RBC) (the latter reflecting Cr(VI)). Genotoxicity was assessed by measurement of micronuclei in peripheral blood reticulocytes (MNRET).

Results: At announced visits, a consistent high degree of compliance to OSH risk prevention measures were seen in 'chromium bath plating' for both technical devices (e.g. ventilation, plastic balls, sheet coverings) and in the use of personal protective equipment (e.g. gloves, respirators), yet a lesser degree of compliance was observed in 'stainless steel welding'. The geometric mean of the air concentration of Cr(VI) was 0.26 μg/m^3 (95% confidence interval (CI): 0.12–0.57) for the Cr(VI)-exposed workers and 3.69 μg/m^3 (95% CI: 1.47–9.25) for the Cr(VI)-exposed apprentices. Subdivided by company type, the exposure levels were 0.13 μg/m^3 (95% CI: 0.04–0.41) for companies manufacturing and processing metal products, and 0.81 μg/m^3 (95% CI: 0.46–1.40) for bath plating companies. Workers with occupational exposure to Cr(VI) had significantly higher median levels of urinary Cr (2.42 μg/L, 5th-95th percentile 0.28–58.39), Cr in RBC (0.89 μg/L, 0.54–4.92) and MNRET (1.59 ‰, 0.78–10.92) compared to the within company controls (urinary: 0.40 μg/L, 0.16–21.3, RBC: 0.60 μg/L, 0.50–0.93,MNRET: 1.06 ‰, 0.71–2.06). When sub-dividing by company type, urinary Cr (4.61 μg/L, 1.72–69.5), Cr in RBC (1.33 μg/L, 0.95–4.98) and MNRET (1.89 μg/L, 0.78–12.92) levels were increased for workers with potential Cr(VI) exposure in bath-plating companies, and when subdividing by work task, workers engaged in

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process operation had increased levels of urinary Cr (8.51 μg/L, 1.71–69.5), Cr in RBC (1.33 μg/L, 0.95–4.98) and MNRET (1.89 μg/L, 0.82–12.92) levels.

Conclusion: This biomonitoring study shows that bath platers were highly exposed to Cr(VI), as suggested by relatively high levels of urinary Cr, Cr in RBC and increased levels of micronuclei. The urinary Cr concentrations were high when compared to the French biological limit value of 2.5 μg Cr/L, corresponding to the Danish occupational exposure limit of 1 μg/m^3. This, in turn, indirectly suggests that additional exposure routes than via air may contribute to the exposure. For welders, no statistically significant increases compared to within company controls were observed, however, the observed urinary Cr levels were similar to the levels observed in a European study (HBM4EU), and were higher than the levels observed for welders in Sweden (SafeChrom). In spite of a high degree of self-reported and observed compliance to OSH risk prevention measures during announced visits, the biomarkers of exposure reflecting recent exposure (urinary Cr) or exposure during the last four months (Cr in RBC) may point to variation in compliance to OSH risk prevention measures in general. Reduced occupational exposure to Cr(VI) may be achieved by applying the hierarchy of controls in eliminating or substituting Cr(VI), and the use of more effective technical solutions (e.g. automation).

Abbreviations

BOEL	Biological occupational limit
CI	Confidence interval
Cr	Chromium
Cr(III)	Trivalent chromium
Cr(VI)	Hexavalent chromium
Cr_{Tot}	Total amount of chromium
DISCO-08	Danish International Standard Classification of Occupation 2008
EU	The European Union
EPA	Environmental Protection Agency
G-EQUAS	German External Quality Assessment Scheme
GM	Geometric mean
GSP	Gesamtstaubprobenahme sampler
HBM4EU	The European Human Biomonitoring Initiative
IARC	International Agency for Research on Cancer
ICP-MS	Inductively coupled plasma mass spectrometry
LEV	Local exhaustion ventilation
LOD	Limit of detection
LOQ	Limit of quantification
MAG	Metal active gas
MIG	Metal inert gas
MMA	Manual metal arc
MNRET	Micronuclei in peripheral blood reticulocytes
NACE	National version of EU's nomenclature
OSH	Occupational safety and health
OEL	Occupational exposure limit
P5	5th percentile
P95	95th percentile
PPE	Personal protective equipment
RBC	Red blood cells
RBC-Cr	Chromium concentration in red blood cells
RPE	Respiratory protective equipment
SOP	Standard operating procedure
TIG	Tungsten inert gas

1. Introduction

Hexavalent chromium (Cr(VI)) is a carcinogen (IARC Group 1) (IARC, 2012). Exposure to Cr(VI) may occur at different industrial processes such as chrome plating and stainless steel welding (IARC, 2012). The main routes of occupational exposure to Cr(VI) are inhalation and dermal contact (IARC, 2012). In addition, hand to mouth contact exposure may result in gastrointestinal tract exposure (Beattie et al., 2017).

In the European Union (EU), occupational exposure to Cr(VI) is regulated both at the European level and by national occupational exposure limits (OEL) in some of the member states. The current EU OEL for Cr(VI) is 10 μg/m^3 (IFA, 2024) which will be further reduced to 5 μg/m^3 in 2025 (Santonen et al., 2022). Sweden has an OEL of 5 μg/m^3 and France, the Netherlands and Denmark have implemented an OEL of 1 μg/m^3 (IFA, 2024). The Danish OEL is expected to be further lowered to 0.25 μg/m^3 in 2025 if deemed technically and economically feasible (Beskæftigelsesministeriet, 2020).

Chromium (Cr) in the urine is often used as a biomarker of internal exposure to Cr(VI) (Verdonck et al., 2021; Viegas et al., 2022). In addition to the regulation regarding airborne exposure to Cr(VI), some European countries have also implemented biological occupational exposure limits (BOELs) using Cr in urine as a biomarker of Cr(VI). France and Finland have implemented BOELs of 2.5 μg/L and 10 μg/L, corresponding to their OELs of 1 and 5 μg/m^3, respectively (Santonen et al., 2022). However, Cr in urine reflects both exposure to Cr(VI) and trivalent chromium (Cr(III)).

The chromium content in the red blood cells (RBC) has been used as a specific marker for the Cr(VI) exposure because only Cr(VI) and not Cr (III) can cross the erythrocyte membrane (Devoy et al., 2016). The content of Cr in the erythrocyte reflects the accumulated exposure over the previous 4 months, corresponding to the lifetime of erythrocytes (Ndaw et al., 2022).

Besides internal exposure biomarkers, some early biological effects can also be assessed from the same human samples, i.e., blood. Flow cytometric analysis of micronuclei in peripheral blood reticulocytes (MNRET) is a sensitive high-throughput method for detection of genotoxicity in biomonitoring studies (Abramsson-Zetterberg et al., 2000). MNRET reflect genotoxicity in bone marrow approximately three days prior to sample collection. The lifespan of human reticulocytes in blood circulation is only 1–4 days and micronucleated reticulocytes are efficiently removed by the spleen. Recently, MNRET was successfully used within the European Human Biomonitoring Initiative (HBM4EU) as a short-term biomarker of genotoxicity from low Cr(VI) exposure, and together with other effect biomarkers it contributed to identifying occupational subgroups that are at increased cancer risk (Tavares et al., 2022). MNRET has also been used for studying the potential genotoxicity of pesticides (Costa et al., 2011), disinfection by-products (Font-Ribera et al., 2019), industrial pollution (Montero-Montoya et al., 2020), and polycyclic aromatic hydrocarbons (Andersen et al., 2021).

The current SAM-Krom biomonitoring study was initiated in response to concern in Denmark regarding the risk of occupational exposure to Cr(VI) in the working environment. The overall purpose of the project was to assess occupational exposure to Cr(VI) and investigate whether occupational safety and health (OSH) risk prevention measures aiming at minimizing workers' exposure to Cr(VI) were present. The present paper describes the results of this Danish Cr(VI) study and adds to a systematic review of biomonitoring data on occupational exposure to Cr(VI) (Verdonck et al., 2021), the recent European HBM4EU Cr(VI) study (Santonen et al., 2022) and the Swedish SafeChrom project (Jiang et al., 2024).

2. Materials and methods

2.1. Study design and study participants

The study is a cross-sectional study carried out in Denmark. The study and the questionnaire were designed to be as similar and comparable as possible to the two recently published chromium studies: 1) the HBM4EU study with workers from eight European countries (Santonen et al., 2022), and 2) the SafeChrom study with workers in Sweden (Jiang et al., 2024). The present study contains: 1) Interview data on company OSH strategy, 2) questionnaire data on self-reported information from workers and apprentices on work tasks, use of personal protective equipment (PPE) and life style habits, 3) data from systematic observations of the use of OSH risk prevention measures, 4) biomonitoring of biomarkers of exposure and effect in blood and urine, as well as personal air exposure measurements carried out in five companies and a vocational school, and 5) personal air exposure measurements on workers from one additional workplace and apprentices/teachers from a vocational school for whom no biological sampling was performed. The vocational school offers both educations with and without Cr(VI) exposure. Thus, apprentices with possible Cr (VI) exposure were recruited for air measurements of Cr(VI) exposure while apprentices in educations without Cr(VI) exposure were recruited at a later stage as within company controls.

2.1.1. Recruitment of companies and processes

In the initial stage of the project, a mapping of existing knowledge on exposure to Cr(VI) in Denmark was performed (Højriis et al., 2020). Based on the mapping and a pilot study performing air measurements at different companies and at different processes, we selected companies and processes meeting the following criteria: 1. The work entails a risk of exposure to Cr(VI) and 2. The work task is present at a larger number of Danish workplaces or exposure occurs repeatedly for the workers involved. Four work tasks were identified: 1) chromium-containing steel welding, 2) production of metal-containing products and work with chromates, 3) thermal spraying, and 4) bath plating (Koponen et al., 2021). Regarding welding type, it was decided to focus on metal active gas (MAG) welding and – to the extent possible – manual metal arc (MMA) welding, while omitting tungsten inert gas (TIG) welding, as the measurements on TIG welding in the pilot study confirmed the assumption that TIG welding does not entail heavy exposure to Cr(VI) due to its low mass emission rate and Cr(VI) conversion factor (Fuglsang et al., 2011; Serageldin and Reeves, 2009).

For the main study, a total number of 29 companies (including four bath-plating companies and 25 companies with manufacturing/processing activities) and one vocational school were contacted by phone. For the companies that met the inclusion criteria and showed immediate interest in the study, this was followed up by an e-mail with more details on the study. Twelve of the 25 manufacturing companies contacted were omitted, as they were only doing TIG welding in stainless steel. Fifteen of the remaining, relevant companies (3 bath plating and 12 manufacture/processing) and the vocational school showed preliminary interest in taking part in the study. However, due to the COVID 19 pandemic, for some of the companies there was a delay of 1–2 years from the initial contact with the companies to the time point when it was possible to perform the measurements. Meanwhile, the COVID 19 pandemic had forced some of the companies to change their production or to reduce their number of workers. Six of the companies (three bath-plating and three manufacture/processing companies) and the vocational school (MMA welding, black smiths and electricians) agreed to participate in the study.

2.1.2. Participants recruited for biomonitoring and personal air exposure assessment

For the biomonitoring part of the study, potentially Cr(VI) exposed participants were recruited from five companies with potential Cr(VI) exposure-related activities such as welding or machining in stainless steel or chromium bath plating. Unexposed participants were recruited from the same companies among office workers with no activities related to Cr(VI) exposure ("within company controls") and from a vocational school with fields of study not involving activities with Cr(VI) exposure ("vocational school controls"). An overview of the recruited exposed and controls stratified by work task on the day the measurement was performed is presented in Table 1.

The biomonitoring study was approved by The Scientific Ethics Committee for the Copenhagen Capital Region (H-20077777). All participants were informed orally and received an information folder about the study before signing a written consent.

2.1.3. Participants only with personal air exposure assessment

In addition to the biomonitoring part of the study, personal air exposure measurements were performed on the following two groups: 1) Apprentices from a stainless steel blacksmith training vocational school, and, 2) Workers from a company with MAG welding activities. The reasons for only performing personal air exposure assessment and no collection of biological samples for this group were that: 1) we did not have an ethical permission for recruiting apprentices for a biomonitoring study at the time of these measurements, and 2) the group at the company consisted almost entirely of non-Danish speaking staff. Our information material accepted by the ethical committee was in Danish.

2.2. Categorisation of companies and work tasks for exposed workers

The companies were categorized according to the Danish Industrial Classification (In Danish "Dansk Branchekode DB07" (Statistics Denmark, 2014) which is a 6-digit classification: "*Dansk Branchekode DB07 is the National version of EU's nomenclature (NACE). The first four digits refer to NACE rev. 2, while the last two represent the Danish subdivision*" (Statistics Denmark, 2014). The SAM-Krom classification of the companies was based on the first 3-digits (Supplementary Table 1).

The job functions for exposed workers were classified according to Statistics Denmark's Classification of Occupations (DISCO-08), v1:2010 (Statistics Denmark, 2010). DISCO-08 is a 6-digit classification, but we chose to use only the 3 first digits for our classification (Supplementary Table 2).

2.3. Interview and questionnaire data

Company leaders with responsibility for OSH were interviewed regarding company OSH strategy, instruction and training, and procedures for handling Cr(VI). On the day of the biological sampling, most participants answered a questionnaire regarding lifestyle habits (e.g. smoking), work tasks, and use of PPE. Questionnaire items included the respondents' use of PPE during various work tasks (chrome [bath] plating; grinding; processing of surfaces with Cr(VI) content; manufacturing of metal products containing Cr(VI); office worker visit to the shop floor) both during the past week and the past three months, and included fresh air fed respirators (independent), powered air purifying respirators (without fresh air), reusable (e.g. half and full face masks) and disposable (single use) respirators, coveralls, gloves and aprons. For tasks involving welding and hard chromium plating/thermal spraying, the PPE included welding helmets with or without respirators as well as fire retardant clothing.

Table 1
Categorisation in SAM-Krom of companies/vocational school (n = 7) and work tasks for individuals (n = 36, exposed group and n = 24, control group) for whom biological and/or air sampling were performed.

Categorisation	Companies	Work task on sampling day																				Total # of individuals			
		Welding				Process operation				Machining				Others				Office work/non-metal work							
	n	EB[a]	E[b]	B[c]	A[d]	EB[a]	E[b]	B[c]	A[d]	EB[a]	E[b]	B[c]	A[d]	EB[a]	E[b]	B[c]	A[d]	EB[a]	E[b]	B[c]	A[d]	EB[a]	E[b]	B[c]	A[d]
Exposed																									
Manufacture/processing of metal products	3	6	3	2	11	0	0	0	0	5	0	0	5	1	0	0	1	0	0	0	0	12	3	2	17
Bath plating	3	0	0	0	0	8	0	1	9	1	0	1	2	0	0	0	0	0	0	0	0	9	0	2	11
Vocational school (stainless steel welding)	1	0	8	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	8
Total		6	11	2	19	8	0	1	9	6	0	1	7	1	0	0	1	0	0	0	0	21	11	4	36
Controls																									
Within company controls: Manufacture/processing of metal products	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5	0	0	5	5
Within company controls: Bath plating	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	6	0	0	6	6
Within company controls: Vocational school (blacksmiths welding and electricians)	1	0	0	9	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	0	0	13	13
Total		0	0	9	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	15	0	0	24	24
Total number of participants																						21	11	28	60

[a] EB: Participants with personal air exposure and biological sampling.
[b] E: Participants only with personal air exposure.
[c] B: Participants only with biological sampling.
[d] A: Participants with any measurement (E or B or (EB).

2.4. Systematic safety observations

Systematic observations of exposure control initiatives were registered in the free app "Safety Observer" (nfa.dk/Safetyobserver), where a tailored list of mandatory chemical exposure control initiatives was drawn up for visual observation during worksite walk-arounds (Kines et al., 2010, 2013; Kirkegaard et al., 2018; Nielsen et al., 2015). The systematic observations were performed by one member of the research team at the same time as the initial biomonitoring measurements. The observation list consisted of Cr(VI) control initiatives in regards to six topics: 1) order and tidiness (workspaces, disposal areas, access and escape routes); 2) physical conditions for working with Cr(VI) (safety signs, labelling, storage, waste); 3) welfare measures (changing, bathing and dining rooms); 4) first aid (signs and supplies); 5) personal protective equipment (airway, skin, eyes, ears); 6) technical devices (ventilation, bubble dispensers, mist suppressants, screens and curtains). One observation marking per object, area (max 50 m^2), machine, tool, person, etc. was scored as either 'correct' or 'not correct'. Notes and photos could be added to the observations. Upon completion of the walk-around in the areas where there were ongoing activities, the app generated a report and a safety index (%) based on the percent of correct observations from the total number of observations.

2.5. Personal air sampling and Cr analysis

Measurement of the inhalable fraction of dust was carried out in accordance with FORCE Technology's accreditation no. 51 from DANAK, and following CEN/TS 15230 (CEN, 2005) and EN 689.

Particles were collected on a PTFE filter (PTFE Membrane Disc Filters - TF 1000, 1 μm, 37 mm) using GSP-3.5 Conical Style Inhalable Dust Samplers. Collection of inhalable dust was carried out with a sample flow of 3.5 l/min. Pumps with automatic regulation for constant flow were used for all measurements, and sample flow was adjusted and calibrated to 3.50 L/min before each measurement.

The air measurements were carried out at each measurement site using two identical parallel sampling systems, and the sampling inlets were both placed in the worker breathing zone outside of any used PPE. One of the two filters exposed in parallel was analyzed for total chromium (Cr_{tot}) and the other for Cr(VI). Blank filters were included for each measurement site and analyzed together with the samples.

Measurements were carried out during normal work activities. The collection time varied based on the duration of the relevant work activities.

Analysis of Cr(VI). Cr(VI) was extracted with a basic EDTA solution to avoid reduction of Cr(VI) to Cr(III). Quantification was based on the addition of the isotope-enriched 50 Cr(VI) using LC-ICP-MS technique. Extraction and analysis was carried out by Eurofins according to USEPA SW-846 METHOD 6800 (2007) (U.S, 2007), and according to Eurofins' DANAK accreditation no. 168. Eurofins reported the relative expanded uncertainty of the analysis to be 20%.The LOD was 0.02 μg/sample, which with an airflow of 3.5 L/min and a minimum sampling time of 2 h, results in a maximum LOD of 0.048 μg/m^3. Thus, the LOD of the air sampling is well below 0.1 μg/m^3, 10% of the Danish OEL.

Analysis of Cr_{Tot}. Extraction and analysis of total chromium followed ISO15202. Chromium and chromium-containing compounds were extracted with strong acid and analysis was carried out by Eurofins by ICP-MS (ISO, 2004). The LOD was 0.2 μg/sample. Eurofins reported the relative expanded uncertainty of the analysis to be 20%.

The calculation of mean, standard deviation etc. was done by including all results below the limit of detection (LOD). This was done by dividing the calculated LOD by 2, and using LOD/2 as the input result in all calculated statistics.

2.6. Blood and urine sample collection

The biological sampling was performed on the same day as the measurement of inhalable Cr_{Tot} and Cr(VI) except for three participants at one of the bath plating companies, where the air measurements were performed approximately five months after the biological sampling for logistical reasons. The blood and spot urine samples were collected on Wednesdays and Thursdays between 10 a.m. and 2 p.m. (after the workers had worked for at least 3–5 h). The participants collected a spot urine sample in an acid-washed cup. Urine samples were transferred to an acid-washed tube. The urine was kept at 4 °C on the sampling day, and stored at −20 °C after transport to NFA. The blood was collected in two vacutainer tubes: 1) one sodium-heparin for micronuclei, 2) one sodium-heparin acid-washed tube for analysis of Cr content. The blood was kept at 4 °C on the sampling day. In the afternoon on the sampling day, after transport to NFA, the erythrocytes were isolated as previously described (Jiang et al., 2024). In brief, the erythrocytes were separated by centrifugation for 10 min at 1300 g and washed three times with isotone saline. The washed erythrocytes were kept at −20 °C until the tubes were used for Cr content analysis. On the day after sample collection, the second sodium-heparin tubes were sent to FIOH for MNRET analysis.

2.7. Measurement of creatinine and density in urine

Cr content in urine was adjusted for urinary dilution in two different ways: 1) density of urine, and 2) creatinine. The density of the urine was measured by weighing. Adjustment of the density was performed according to (Jiang et al., 2024) using the following formula: $C_{(density\text{-}adjusted)} = C \times (1\text{-}\rho_{mean})/(1\text{-}\rho_{sample\ density})$, where C = the determined Cr concentration in the sample, ρ_{mean} = the mean of the urinary density of all participants, and $\rho_{sample\ density}$ = the density of the urine sample. The urine density of three samples was below 1.00 g/mL suggesting that the urine had been diluted with water. The samples were excluded from the analysis of density-adjusted Cr content in urine. Creatinine was measured as previously described (Hansen et al., 2008). The molecular weight of creatinine (113.1 g/mol) was used.

2.8. Measurements of Cr in urine and red blood cells

Tubes and tips were washed with acid (5% HCl and 5% HNO_3) to remove metal background contamination. Analysis of Cr content in urine and blood was performed essentially according to (Jiang et al., 2024). The samples were stored at −4 °C prior to the analysis and thawed at room temperature on the day of analysis. Sample volumes of 250 μL (urine) or 100 μL (blood) were diluted 20 times in disposable polypropylene tubes (Sarstedt AG & Co. KG, Germany) with an alkaline solution containing 0.5 g/L EDTA disodium salt dihydrate (Merck, Darmstadt, Germany), 0.5 g/L Triton X-100 (Sigma-Aldrich, St Louis, USA) and 5 g/L ammonia (25 %, suprapur, Merck KGaA, Darmstadt, Germany). The samples were prepared in duplicates (R1 and R2). The mean values were used in subsequent statistical analyses.

The total mass concentration of Cr in the samples was determined by inductively coupled plasma-mass spectrometry (ICP-MS) using an iCAP TQ ICP-MS (Thermo Fisher Scientific, Bremen, Germany) equipped with an ASX-560 autosampler and a ASXpress PLUS valve equipped with the 1 mL sample loop (Teledyne CETAC Technologies, Omaha, NE, USA). The analysis was performed in single quadrupole mode with helium as collision gas in the collision cell (kinetic energy discrimination). Instrumental configuration and parameters are listed in Supplementary Table 3.

For matrix matching of the calibration standards, whole blood (SERONORM, SERO AS, Billingstad, Norway Whole blood L-1, Lot: 2011920, Cr: 0.61 μg/L) and urine (The German External Quality Assesment Scheme, Erlangen Germay) (G-EQUAS R64/2019 8 A, Cr: 0.25 ± 0.09 μg/L, were prepared in the same way as the samples and spiked with Cr concentrations of 0.5, 1, 5 and 10 μg/L.

Internal standard correction was performed by spiking 1 μg/L rhodium (Rh) to all samples and calibration standards. Calibration and internal standard were prepared from standard solutions that contained 1000 mg/L of Cr or Rh (PlasmaCAL, SCP Science, Baie D'Urfé, QC, Canada).

The analytical accuracy was verified towards certified reference materials from G-EQUAS and Seronorm. The results (μg/L, mean ± SD) obtained for G-EQUAS (Lot. R64/2019 1 A) were for Cr in blood 2.0 ± 0.1 vs. range 1.1–2.3 and for Seronorm (Lot, 2011920) in blood 0.70 ± 0.12 vs. range 0.48–0.75. For G-EQUAS Cr in urine (Lot. R64/2019 2 A and 8 A) the results obtained were 3.5 ± 0.1 vs. range 2.8–4.0 and 0.22 ± 0.02 vs. range 0.16–0.34 respectively. The relative standard deviation based on triplicate analysis of the certified reference materials was 3 % for urine and 3.4% for blood (G-EQUAS).

The limit of detection (LOD) and limit of quantification (LOQ), calculated as 3- and 10-times the standard deviation of the blank samples, were 0.05 and 0.16 μg/L, respectively, for the urine and 0.2 and 0.6 μg/L, respectively, for the RBC.

2.9. Analysis of micronuclei frequency

Chromosomal damage was assessed by MNRET as previously described (Andersen et al., 2021; Tavares et al., 2022). Briefly, 2 mL whole blood samples collected with sodium-heparin were stored at 4 °C and processed within seven days after collection. Immunomagnetic bead separation was performed according to the instructions of the CELLection™ Pan Mouse IgG Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) to isolate transferrin-positive (+CD71) reticulocytes using a FITC Mouse Anti-human CD71 antibody (BD Biosciences, San Jose, CA, USA). Samples were fixed in 2% paraformaldehyde in PBS with 10 μg/mL of sodium dodecyl sulfate (SDS; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) and stored refrigerated (4 °C) until analysis. Prior to the analysis, each sample was divided into two replicates and DNA was stained with Hoechst 33 342 (Invitrogen, Thermo Fisher Scientific, Walthamt, MA, USA). The samples were analyzed with CytoFlex S flow cytometer (Beckman Coulter, Brea, CA, USA) using blue (488 nm) laser for the identification of +CD71 reticulocytes and near UV (375 nm) laser for the detection of DNA-containing micronuclei. CytExpert software version 2.3 was used for data acquisition and analysis of 20 000–150 000 +CD71 reticulocytes per replicate sample. The micronuclei frequency was quantified as per-mille (‰) of micronucleated reticulocytes from all analyzed + CD71 reticulocytes.

2.10. Statistical analysis

Statistical testing was performed using GraphPad Prism 8.0.2. For continuous variables, statistically significant differences between exposed and controls were determined using the Kruskal–Wallis test with Dunn's post-hoc. Statistically significant differences between exposed stratified by company and work task and controls were determined using Kruskal-Wallis test with Dunn's post-hoc. For categorical variables, the Chi-square test and Fisher's exact test were used to compare differences between exposed and controls. Correlations between variables were assessed by Spearman's correlation.

3. Results

3.1. Characteristics of the study population

In total, the study involved 60 individuals including 36 classified as Cr(VI) exposed and 24 classified as controls. The numbers of participants with biological sampling and/or personal air exposure assessment across different company types/vocational schools and work tasks are shown in Table 1.

The volunteers from the exposed group were recruited from six companies (three companies performing manufacture/processing of metal products and three companies performing bath plating) and one vocational school, where apprentices were taught to perform stainless steel welding. The exposure measurements and biological sampling were performed between January 2022 and October 2023.

The volunteers from the control group (n = 24) were recruited from the same six companies and the same vocational school as the exposed group. At the companies, office workers were recruited, and at the vocational school, apprentices at other educations (electricians, blacksmiths welding) were recruited. Stationary exposure measurements at relevant locations within the primary working area of the controls were used as proxies for the personal air exposure for the control group.

The characteristics of the 49 participants (25 exposed and 24 controls) with biological samples are presented in Table 2.

Both types of controls (within company controls from the six companies and vocational school controls) were regarded as within company controls, as both are in occupational settings with possible bystander exposures to Cr(VI). The two types of within company controls differed from the group of Cr(VI) exposed for some lifestyle characteristics (results not shown), but much less, when the two types of controls were merged into one group. For this reason, and to increase the statistical power, the two types of controls were merged into one within company control group.

3.2. Interviews, questionnaire and observations regarding OSH risk prevention

In the interviews with OSH leaders from the six companies (three bath plating, three manufacture/processing of metal products) and one vocational school), all were highly aware of the OSH risks of working with Cr(VI), and had either written or verbal procedures for handling and working with Cr(VI). Some collaborated with external OSH experts in carrying out yearly internal OSH meetings, establishing standard operating procedures (SOPs), carrying out regular air measurements, etc. Onboarding, instruction and training in Cr(VI) work tasks were most often done through a learning-by-doing 'buddy' system – although most workers had received some general OSH vocational training. Company sizes (micro to large) and the degree of systematic OSH work varied greatly, from a very informal verbal culture, to one company having attained the ISO4500 international standard for their formal OSH management system.

In regards to use of PPE and local exhaust ventilation systems, questionnaire responses were received from 9 of the 11 bath plating respondents, 12 of the 14 metal manufacturing respondents (welding and grinding) and 9 of the 13 apprentices serving as within company controls. For the bath plating workers (in both the previous week and previous three months) the most often used form of respiratory protection were disposable respirators (single use P2/P3 masks), followed by powered air purifying respirators (dependent, without fresh air). Interviews with the OSH leaders and systematic observations of the work processes revealed that all three bath plating companies used local exhaust ventilation systems attached to the bath plating equipment, and also used plastic balls and plastic sheets or cardboard coverings to reduce aerosol generation for most of the baths. In the three welding and grinding companies and the vocational school education entailing potential Cr(VI) exposure, they all used local exhaust ventilation with moveable capture hoods, and the workers/apprentices primarily used welding helmets without further respiratory protection. However, two of the workers, who did not fill out the questionnaire, were observed using air-fed (independent) respirators. Safety observation indexes were relatively high in both the three bath plating cases (94% average) and four welding/grinding cases (86% average). The lower indexes in the welding/grinding cases were partly due to incorrect use of the moveable capture (ventilation) hoods. Appropriate gloves for the various purposes (e.g. welding, bath plating) were worn by all workers, and coveralls/aprons were worn by most workers. The latter were used over a period of time (shifts) before being washed or replaced.

Table 2
Characteristics of the study group in Sam-Krom.

	Cr(VI) Exposed n = 25	Controls n = 24	*P*
Age, median (P5, P95)	50 (26.3, 66.8)	53 (18.5, 69.8)	0.69[a]
Female, n (%)	0 (0)	4 (16.7)	0.03[b]
Body Mass Index, median (P5, P95)	27.0 (17.5, 46.0)	28.0 (18.6, 58.4)	0.80[a]
Smoking, n (%)			0.50[c]
Never smoker	11 (44)	10 (42)	
Previous smoker	4 (16)	7 (29)	
Current smoker	10 (40)	7 (29)	
Coffee drinking, n (%)	22 (88)	18 (75)	0.24[b]
Tea drinking, n (%)	6 (24)	8 (33)	0.47[b]
Diet (mix, vegetarian, vegan), n (%)	25/0/0 (100/0/0)	24/0/0 (100/0/0)	d
Supplement, n (%)	8 (32)	6 (25)	0.59[b]
Implant, n (%)	5 (20)	5 (21)	>0.99[b]
Leisure activity with Cr, n (%)	4 (16)	0 (0)	0.04[b]

[a] Mann-Whitney test.
[b] Chi-square test.
[c] Fisher's exact test.
[d] No statistical testing, all participants are on a mixed diet.

3.3. Cr(VI) in air

Concentrations of inhalable Cr(VI) measured in the exposed group and stratified by company and work task are shown in Table 3 and Fig. 1. The geometric mean of the air concentration of Cr (VI) was 0.26 μg/m^3 for the workers with potential Cr(VI) exposure and 3.69 μg/m^3 for the apprentices with potential Cr(VI) exposure. When subdividing by company type, the exposure levels were 0.13 μg/m^3 (95% CI: 0.04–0.41) for companies manufacturing and processing of metal products, 0.81 μg/m^3 (95% CI: 0.46–1.40) for bath plating companies and 3.69 μg/m^3 (95% CI: 1.47–9.25) at vocational schools during welding education.

For the workers with potential Cr(VI) exposure, 19 out of 24 (79%) personal air measurements of inhalable Cr(VI) were below the Danish OEL of 1 μg/m^3 while 10 out of 24 (42%) measurements were below the expected future Danish OEL of 0.25 μg/m^3 (Fig. 2).

When subdividing by company type, two of fifteen air measurements for companies in manufacturing and processing of metal products exceeded the Danish OEL of 1 μg/m^3, as did 3/9 measurements for bath plating companies and 6/8 measurements during welding exercises at the vocational school. Five of fifteen air measurements for companies in manufacturing and processing of metal products exceeded the expected future Danish OEL of 0.25 μg/m^3, as did 9/9 measurements for bath plating companies and 8/8 measurements during welding exercises at the vocational school.

3.4. Cr in urine

Urine content of Cr was used as a biomarker of recent exposure to Cr (VI), while bearing in mind that urinary Cr reflects exposure to Cr(III) and Cr(VI). Urinary Cr content is presented in three different ways as Cr content in urine, Cr content normalised to creatinine, and density adjusted (Table 4). Urinary Cr was increased in workers with potential Cr (VI) exposure as compared to controls for all three measures of urinary Cr (Table 4 and Fig. 3). Thus, workers with potential occupational exposure to Cr(VI) had a median Cr of 1.52 μg Cr/g crea (5th-95th percentile 0.25–100) as compared to the controls with a median of 0.31 μg Cr/g crea (0.14–4.1). Urinary Cr levels did not differ between within company controls and vocational school controls (results not shown). When sub-dividing by company or work tasks, urinary Cr was increased for workers with potential Cr(VI) exposure at bath-plating companies (median 3.12 μg Cr/g crea (1.72–137.1) compared to controls and when subdividing by work task, workers engaged in process operation had significantly increased urinary Cr levels (median Cr level: 3.95 μg Cr/g crea (1.72–137.1) μg Cr/g crea) compared to controls.

The French BOEL of 2.5 μg/L Cr was exceeded for 9 out of 11 workers

Table 3

Upper panel: concentrations of inhalable total chromium (Cr_{Tot}; µg/m^3) and hexavalent chromium (Cr(VI); µg/m^3) measured in the exposed group (vocational school apprentices and workers) and within company controls and stratified by company and work task. Lower panel: stationary measurements of CrTot and Cr(VI) at control locations and locations with potential Cr exposure at the companies.

AIR (PERSONAL)		Controls[c]	Vocational school Apprentices exposed[d]	Workers exposed[e]	COMPANY - TYPE[a]		COMPANY - WORK TASK[b]		
					Manufacture/processing[f]	Bath plating[f]	Welding[g]	Process operation[g]	Machining[g]
Cr(VI)	n (n < LOD)[m]		8 (0)	24 (5)	15 (5)	9 (0)	9 (1)	8 (0)	6 (3)
	GM (95% CI)		3.69 (1.47; 9.25)	0.26 (0.12; 0.57)	0.13 (0.04: 0.41)	0.81 (0.46; 1.40)	0.24 (0.05; 1.28)	0.86 (0.47; 1.60)	0.09 (0.01; 0.53)
	Median (P5, P95)		3.80 (0.80; 20.0)[q]	0.42 (0.02; 21.74)[q]	0.08 (0.02; 27.96)[n]	0.71 (0.28; 3.10)[p]	0.22 (0.02; 28.96)[n]	0.79 (0.28; 3.10)[q]	0.06 (0.02; 1.01)
	Mean (95% CI)		5.98 (0.69; 11.26)	1.69 (−0.70; 4.07)	2.08 (−1.89; 6.05)	1.03 (0.36; 1.70)	3.33 (−3.77; 10.43)	1.10 (0.35; 1.86)	0.27 (−0.15; 0.69)
Cr_{Tot}	n (n < LOD)[m]		8 (0)	24 (1)	15 (0)	9 (1)	9 (6)	8 (1)	6 (0)
	GM (CI)		9.99 (5.09; 19.61)	4.49 (2.46; 8.16)	6.86 (3.50; 7.03)	2.21 (0.69; 7.10)	12.87 (5.74; 28.88)[n]	1.67 (0.54; 5.31))	3.85 (1.24; 12.01)[n]
	Median (P5, P95)		11.85 (2.70; 31.40)[q]	4.03 (0.28; 61.08)[q]	7.47 (1.60; 61.40)[q]	2.29 (0.25; 19.20)[n]	11.20 (3.00; 61.40)[q]	1.95 (0.25; 9.10))	2.55 (1.60; 19.20)
	Mean (95% CI)		12.95 (4.95; 20.95)	10.76 (3.82; 17.69)	14.14 (3.23; 25.05)	5.11 (0.27; 9.95)	21.17 (3.44; 38.89)	3.35 (0.29; 6.41)	6.45 (−1.17; 14.07)
AIR (STATIONARY)		Control location[h]	Vocational school[i]	Cr companies[j]	Manufacture/processing[k]	Bath plating[k]	Welding[l]	Process operation[l]	Machining[l]
Cr(VI)	n (n < LOD)[m]	11 (9)		8 (1)	3 (1)	5 (0)			
	GM (95% CI)	0.017 (0.01; 0.03)		0.22 (0.05; 0.90)	0.05 (0.00; 4.56)	0.53 (0.18; 1.54)			
	Median (P5, P95)	0.011 (0.01; 0.13)		0.30 (0.011; 1.28)	0.01; 0.36)	0.82 (0.19; 1.28)[o]			
	Mean (95% CI)	0.03 (0.002; 0.05)		0.48 (0.09; 0.87)	0.13 (−0.35; 0.62)	0.69 (0.11; 1.26)			
Cr_{Tot}	n (n < LOD)[m]	11 (7)		8 (1)	3 (0)	5 (1)			
	GM (CI)	0.35 (0.20; 0.59)		1.73 (0.61; 4.89)	3.03 (0.11; 83.85)	1.23 (0.28; 5.47)			
	Median (P5, P95)	0.39 (0.10; 1.60)		1.40 (0.25; 14.18)[n]	1.40 (1.40; 14.18)	1.01 (0.25; 5.00)			
	Mean (95% CI)	0.46 (0.18; 0.75)		3.39 (−0.47; 7.26)	5.66 (−12.67; 23.99)	2.03 (−0.46; 4.53)			

[a] Endpoints stratified by company type.
[b] Endpoints stratified by company work task. Results from "Others" not shown for GDPR reasons (only one participant).
[c] No personal air measurements performed among controls.
[d] Stars indicates statistically significance following a Mann-Whitney test, stationary control measurements versus vocational school.
[e] Stars indicates statistically significance following a Mann-Whitney test, stationary control measurements versus all company exposed.
[f] Stars indicate statistically significance by Dunnet's multiple comparisons test, stationary control measurements versus company types, as a post hoc analysis following a significant Kruskal-Wallis test.
[g] Stars indicate statistically significance by Dunnet's multiple comparisons test, stationary control measurements versus work tasks, as a post hoc analysis following a significant Kruskal-Wallis test.
[h] Stationary air measurements performed at control locations (companies and vocational school).
[i] No stationary measurements were performed in exposed locations on the vocational school.
[j] Stars indicates statistically significance following a Mann-Whitney test, stationary control measurements versus all company exposed.
[k] Dunnet's multiple comparisons test, controls versus work tasks, as a post hoc analysis following a significant Kruskal-Wallis test.
[l] No measurements.
[m] Number of measurements (number of measurements below LOD.
[n] $p < 0.05$.
[o] $p < 0.01$.
[p] $p < 0.001$.
[q] $p < 0.0001$.

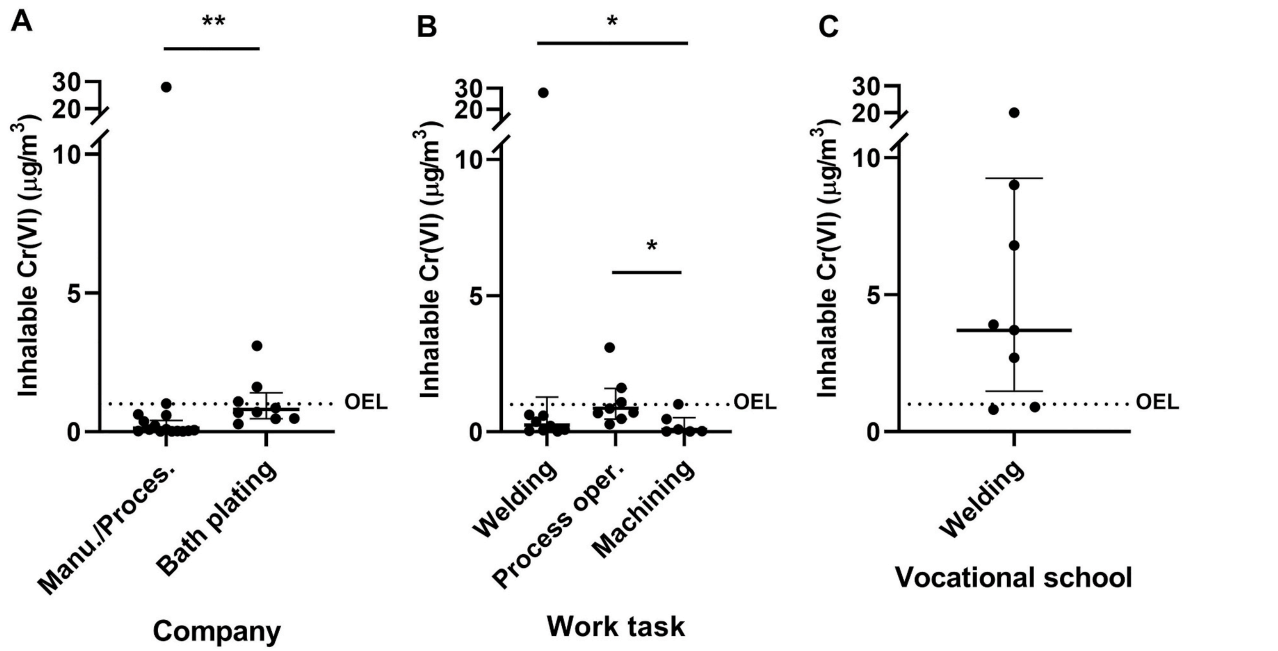

Fig. 1. Inhalable hexavalent chromium (Cr(VI)) in exposed workers across company and work task and in exposed students at a vocational school
A. Inhalable hexavalent chromium (Cr(VI)) in exposed workers across company; B. Inhalable hexavalent chromium (Cr(VI)) in exposed workers across work task; C. Inhalable hexavalent chromium (Cr(VI)) in exposed apprentices at a vocational school; OEL: Occupatinal exposure limit, Manu./Proces: Manufacture/Processing of metal products, Process oper.: Process operation; Dunn's post-hoc test, **P* < 0.05, ***P* < 0.01. The data are presented as geometric mean and 95% CI.

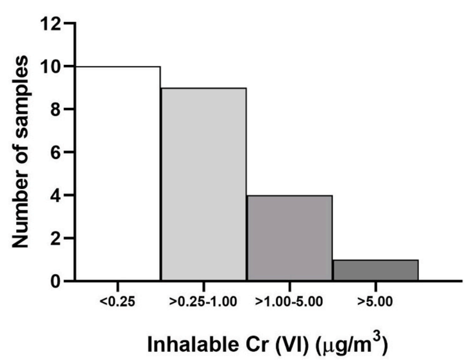

Fig. 2. Frequency distribution histogram for personal measurements of inhalable Cr(VI) at companies.

with potential Cr(VI) exposure at bath plating companies, for 2 out of 14 workers within manufacture and processing and for one out of 24 within-company control. The Finnish BOEL of ca. 10 μg/L Cr was exceeded for 3 out of 11 workers with potential Cr(VI) exposure at bath plating companies, for none of the workers within manufacture and processing and for one out of 24 within-company control.

3.5. Cr in red blood cells

Cr in RBC was used as a biomarker of the cumulative exposure to Cr (VI) the last 4 months (Table 4). Cr(VI) content in RBC was increased in workers with potential Cr(VI) exposure (median level: 0.89 μg/L (95% CI: 0.54–4.9) as compared to controls (median: 0.60 (0.50–0.93) (P < 0.0001). Levels of Cr(VI) in red blood cells did not differ between within company controls and vocational school controls (results not shown). When subdivided by company type and work task, workers with potential Cr(VI) exposure at bath plating companies had significantly increased blood levels of Cr(VI) (P < 0.0001), as had process operators (P < 0.0001) when subdivided by work task (Fig. 4 and Table 4).

3.6. Micronuclei

MNRET was used as a biomarker of recent genotoxic exposure. The median MNRET frequency was 1.59 (5th-95th percentile: 0.78; 10.92) in Cr(VI) exposed and 1.06 (0.72; 2.064) in within company controls (Table 4). When subdividing by company or work task (Fig. 4), Cr (VI)-exposed workers at bath plating companies had increased median levels of MNRET (1.89 (0.78–12.92)) as compared to controls (1.06 (0.71–2.06) (p = 0.004), while the level among Cr(VI) exposed workers at manufacture/processing companies (1.31 (0.79–2.58) was not increased compared to controls. Similarly, subdividing by work task revealed that the median level of MNRET for the work task process operation (1.89 (0.82–12.92)) was significantly increased compared to controls. The medians for work tasks welding (1.49 (0.79–2.38)) and machining (0.95 (0.78–6.25)) were not significantly increased compared to controls.

3.7. Correlation between effect and exposure biomarkers

Correlations between exposure and effect biomarkers are presented in Fig. 5 and Supplementary Fig. 1. There were strong correlations between inhalable Cr(VI) and the urinary Cr (unadjusted: Spearman's rank correlation coefficients (r_S) = 0.75; creatinine adjusted: r_S = 0.71 and density adjusted: r_S = 0.76)). Inhalable Cr(VI) correlated moderately with the Cr content in RBC (r_S = 0.52). There were also strong to very strong correlations between RBC-Cr and all three ways of presenting urinary Cr (unadjusted: r_S = 0.77; creatinine adjusted: r_S = 0.80 and density adjusted: r_S = 0.76). The effect biomarker for genotoxicity, MNRET, correlated moderately to the creatinine adjusted urinary Cr content (r_S = 0.40) and moderately and negatively to Cr_{Tot} in air (r_S = -0.51).

4. Discussion

This is the first study on Cr(VI) exposure and toxicity in Denmark in 30 years and shows that although the Cr(VI) levels in air have decreased, particularly bath platers still have an elevated Cr(VI) exposure. Overall,

Table 4
Chromium (Cr) concentration in urine and red blood cells (RBC) and micronucleus assay in reticulocytes (MNRET).

URINE		Unit	Controls[c]	Workers exposed[d,e]	COMPANY[a]		WORK TASK[b]		
					Manufacture/processing[f]	Bath plating[g]	Welding	Process operation[h]	Machining
Cr	**Unadjusted**	**µg Cr/L**	**n=24**	**n=25**	**n=14**	**n=11**	**n=8**	**n=9**	**n=7**
	Median (P5, P95)		0.40 (0.05; 6.44)	1.47 (0.2; 50.7)[m]	0.56 (0.14; 3.91)	4.68 (1.47; 64.6)[n]	0.55 (0.10; 2.30)	4.70 (1.50; 64.6)[n]	0.90 (0.40; 5.0)
	Mean (95% CI)		0.78 (0.10; 1.46)	5.66 (0.29; 11.0)	0.94 (0.36; 1.52)	11.69 (−059; 23.96)	0.80 (0.22; 1.38)	13.51 (−1.78; 28.8)	1.89 (0.19; 3.59)
	Creatinine adjusted	**µg Cr/g crea**	**n=24**	**n=25**	**n=14**	**n=11**	**n=8**	**n=9**	**n=7**
	Median (P5, P95)		0.31 (0.14; 4.1)	1.52 (0.25; 100)[m]	0.50 (0.20; 2.0)	3.12 (1.72; 137.1)[n]	0.65 (0.44; 2.0)	3.95 (1.72; 137.1)[n]	0.95 (0.38; 3.1)
	Mean (95% CI)		0.62 (0.19; 1.04)	7.74 (−3.46; 18.93)	0.78 (0.48; 1.07)	16.6 (−10.37; 43.56)	0.87 (0.42; 1.32)	19.7 (−14.3; 53.7)	1.3 (0.32; 2.23)
	Density adjusted[i]	**µg Cr/L**	**n =22**	**n=24**	**n=13**	**n=11**	**n=7**	**n=9**	**n=7**
	Median (P5, P95)		0.40 (0.16; 21.3)	2.42 (0.28; 58.39)[m]	0.69 (0.28; 5.89)	4.61 (1.72; 69.5)[l]	1.15 (0.35; 5.89)	8.51 (1.71; 69.5)[l]	0.69 (0.30; 4.21)
	Mean (95% CI)		1.72 (−0.57; 4.00)	6.98 (0.85; 13.10)	1.41 (0.41; 2.42)	13.55 (0.23; 26.87)	1.79 (0.03; 3.56)	15.80 (−0.69; 32.32)	1.77 (0.20; 3.34)
BLOOD		Unit	Controls[c]	Workers exposed[d,e]	Manufacture/processing[f]	Bath plating[g]	Welding	Process operation[h]	Machining
RBC-Cr		**µg Cr/L**	**n=24**	**n=25**	**n=14**	**n=11**	**n=8**	**n=9**	**n=7**
	Median (P5, P95)		0.60 (0.50; 0.93)	0.89 (0.54; 4.92)[m]	0.71 (0.52; 1.00)	1.33 (0.95; 4.98)[n]	0.74 (0.52; 1.00)	1.33 (0.95; 4.98)[n]	0.84 (0.59; 1.43)
	Mean (95% CI)		0.63 (0.58; 0.68)	1.41 (0.86; 1.96)	0.72 (0.65; 0.80)	2.28 (1.16; 3.41)	0.74 (0.63; 0.85)	2.49 (1.11; 3.88)	0.89 (0.57; 1.20)
MNRET[j]		‰	**n=24**	**n=25**	**n=14**	**n=11**	**n=8**	**n=9**	**n=7**
	Median (P5, P95)		1.06 (0.71; 2.06)	1.59 (0.78; 10.92)[k]	1.31 (0.79; 2.58)	1.89 (0.78; 12.92)[l]	1.49 (0.79; 2.38)	1.89 (0.82; 12.92)[l]	0.95 (0.78; 6.25)
	Mean (95% CI)		1.14 (0.99; 1.30)	2.27 (1.22; 3.32)	1.42 (1.07; 1.76)	3.35 (0.96; 5.74)	1.41 (0.98; 1.84)	3.32 (0.45; 6.18)	2.06 (0.22; 3.90)

P5: 5th percentile; P95: 95th percentile; 95% CI: 95% confidence interval.
[a] Endpoints stratified by company type.
[b] Endpoints stratified by company work task. Results from "Others" not shown for GDPR reasons (only one participant).
[c] All controls (within company controls + vocational school controls).
[d] All company exposed.
[e] Stars indicates statistically significance following a Mann-Whitney test, controls versus all company exposed.
[f] Manufacture/procession used as abbreviation of Manufacture/procession of metal products.
[g] Stars indicate statistically significance by Dunnet's multiple comparisons test, controls versus company types, as a post hoc analysis following a significant Kruskal-Wallis test.
[h] Stars indicate statistically significance by Dunnet's multiple comparisons test, controls verus work tasks, as a post hoc analysis following a significant Kruskal-Wallis test.
[i] Three samples were taken out due to a negative density (2 from the control group and 1 from the exposed group).
[j] Chromosomal damage assessed by the micronucleus assay in reticulocytes (MNRET).
[k] $p < 0.05$.
[l] $p < 0.01$.
[m] $p < 0.001$.
[n] $p < 0.0001$.

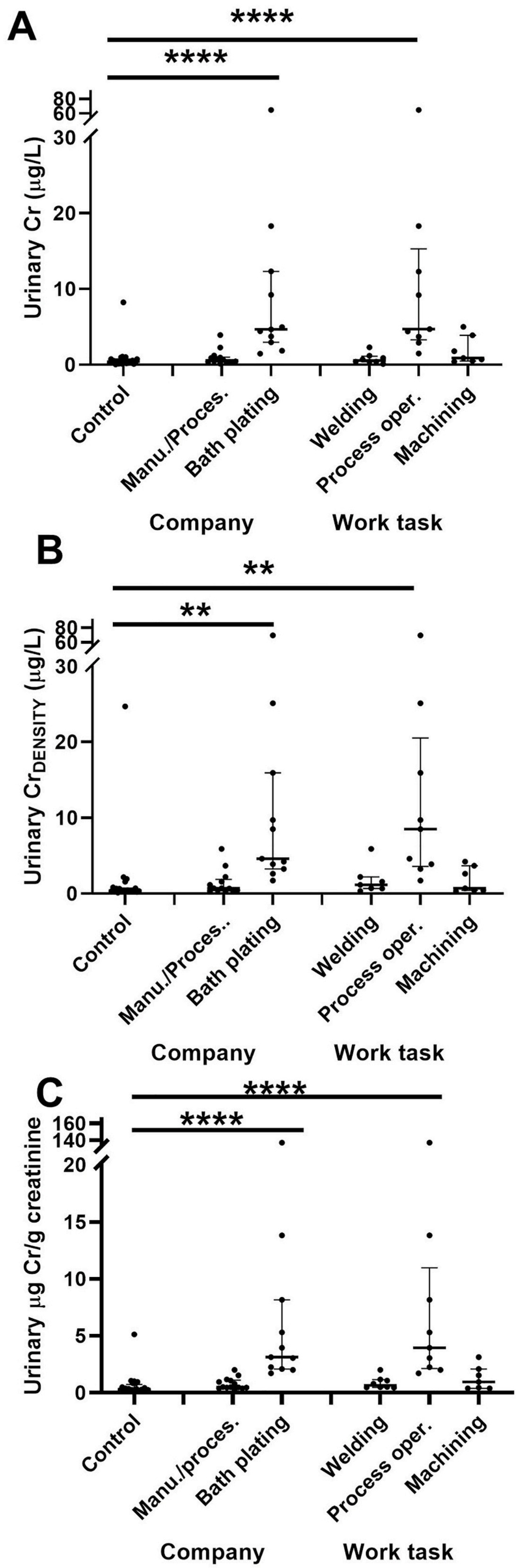

Fig. 3. Urinary Cr in controls and exposed workers across company and work task

A. Urinary Cr (unadjusted); B. Urinary Cr (density adjusted); C. Urinary Cr (creatinine adjusted); Manu./Proces: Manufacture/Processing of metal products, Process oper.: Process operation; Dunn's post-hoc test, $**P < 0.01$, $****P < 0.0001$. The data are presented as median and interquartile range.

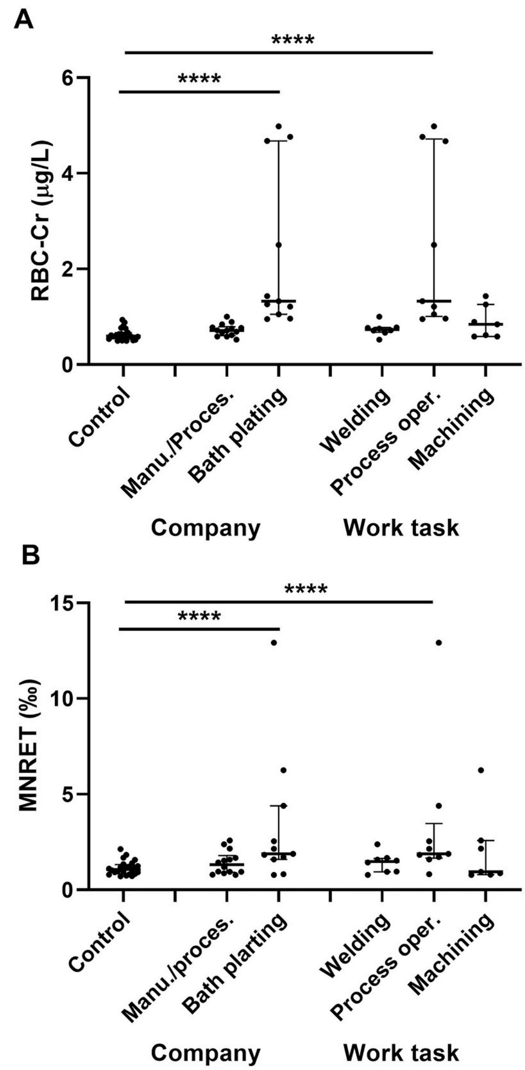

Fig. 4. Red blood cell Cr (RBC-Cr) and micronuclei in peripheral blood reticulocytes (MNRET) in controls and exposed workers across company and work task

A. RBC; B. MNRET; Manu./Proces: Manufacture/Processing of metal products, Process oper.: Process operation; Dunn's post-hoc test, $****P < 0.0001$. The data are presented as median and interquartile range.

the SAM-Krom biomonitoring study recruited 60 subjects including 36 with possible occupational exposure to Cr(VI) and 24 without occupational exposure to Cr(VI). The company types included were selected based on a report reviewing the current knowledge on occupational exposure to Cr(VI) in Denmark (Højriis et al., 2020). The report concluded that manufacturing and processing was the company category with the highest number of workers with possible Cr(VI) exposure, while bath plating represented companies with possible high occupational exposure to Cr(VI) (Højriis et al., 2020). The report estimated that there are 5–7 Danish bath plating companies with 10–49 workers with possible occupational exposure to Cr(VI) and ca. 5.000–20.000 workers in welding, thermal cutting and sanding in stainless steel who are potentially occupationally exposed to Cr(VI) (Højriis et al., 2020). For welding, we recruited companies doing MAG and MMA welding in stainless steel as these are the primary types of welding entailing Cr(VI) exposure (Højriis et al., 2020). We included three bath-plating companies encompassing 11 participants with possible occupational exposure to Cr(VI) and 6 within company controls. Thus, the SAM-Krom study included a substantial fraction of the bath-plating companies and

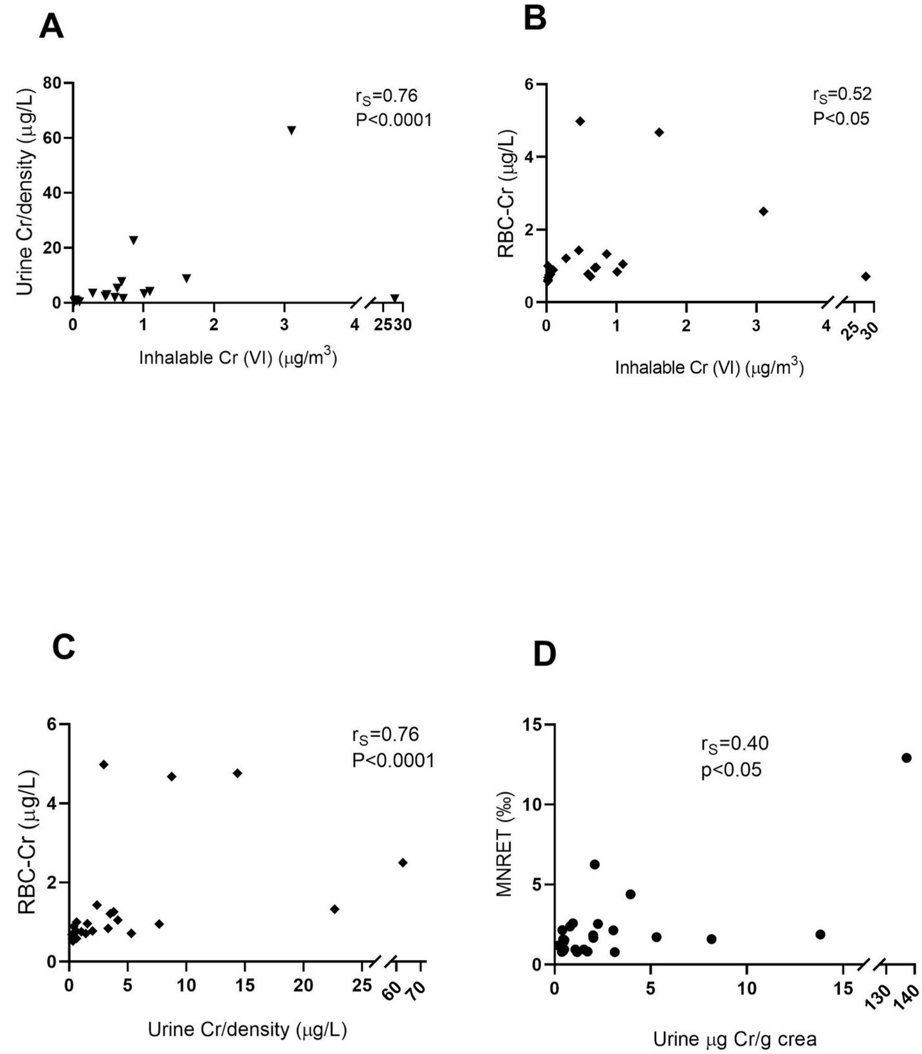

Fig. 5. Scatter plot and Spearman correlation analysis between inhalable Cr(VI), red blood cells Cr (RBC-Cr), urine Cr/density, urine Cr/Crea and micronuclei in peripheral blood reticulocytes (MNRET) in the exposed group.
A. Urine Cr/density versus inhalable Cr(VI); B. RBC-Cr versus inhalable Cr(VI); C. RBC-Cr versus urine Cr/density; D. MNRET versus Urine Cr/Creatinine.

bath platers in Denmark. In Supplementary Table 4, results from SAM-Krom are compared to the Swedish SafeCrom and the European HBM4EU results.

4.1. Cr(VI) in air

Air concentrations of Cr(VI) were measured for 3.0 h on average, thus reflecting 37.5% of an 8-h work day. Assuming that the air measurements are representative for an 8-h working day, the air concentration of Cr(VI) in the working areas of the within company controls was low (0.017 μg/m^3, (95% CI: 0.01; 0.03) and most of the measurements were below the limit of detection. For workers with possible occupational exposure to Cr(VI), the geometric mean for Cr(VI) exposure was significantly increased and more than 10 times higher; 0.26 μg/m^3, (95% CI:0.12; 0.57), while still below the Danish OEL. When subdiving by company type, bath plating companies had significantly higher Cr(VI) concentrations in the air (geometric mean (GM): 0.81 μg/m^3 (95% CI: 0.46; 1.40) as compared with within company offices and as compared to air levels within manufacturing (GM: 0.13 μg/m^3 (95% CI: 0.04: 0.41). We measured rather high Cr(VI) exposure levels (GM: 3.69 μg/m^3, (95% CI: 1.47; 9.25) for trainees performing MMA welding at a vocational school, highlighting the need to increase the focus on OSH risk preventive measures and training in using the preventive measures in the education and training processes.

Knudsen et al. (1992) performed a biomonitoring study of occupational exposure to Cr(VI) among 127 welders and 80 reference persons in Denmark in 1987 (Knudsen et al., 1992). Knudsen et al. reported air exposure levels to chromates as geometrical means of 0.9 μg/m^3 (0.4 μg Cr(VI)/m^3), 1.4 μg/m^3 (0.6 μg Cr(VI)/m^3) and 1.5 μg/m^3 (0.7 μg Cr (VI)/m^3) for TIG, MMA + TIG, and metal inert gas (MIG) welders, respectively, thus reporting 2–3 times higher Cr(VI) air concentrations than in the current study (GM for welding: 0.24 μg/m^3 (95% CI: 0.05; 1.28). Bonde and Christensen (1991) assessed Cr exposure in 60 welders and 45 reference persons at Danish workplaces and reported median values of time-weighted average exposure to Cr(VI) in air (Bonde and Christensen, 1991). For TIG welders in stainless steel, Cr(VI) was 3 μg/m^3 and for mild steel welding, 1 μg/m^3 was reported. The corresponding median Cr(VI) air concentration for welders in the current study was 0.2 μg/m^3, (95% CI: 0.02; 28.96). Thus, the occupational exposure levels to Cr(VI) for welders seem to be lower as compared with the two more than 30-year-old Danish studies on welders. When comparing to contemporary studies, the Swedish SafeChrom study, reported similar levels as in the present study: GM for welders as 0.17 μg/m^3, (95% CI: 0.08–0.37) and the median 0.1 μg/m^3, (P5-95: 0.02–14.73) (Jiang et al., 2024). In the large HBM4EU study, GM for Cr (VI) measured outside any respiratory protective equipment (RPE) was

0.5 μg/m^3 and median air concentration was also 0.5 μg/m^3.

In the current study, Cr(VI) exposure levels for the work task process operation, representing bath plating was GM: 0.86 μg/m^3, (95% CI: 0.47; 1.60). In SafeChrom, process operation (representing bath plating) had GM of 0.19 μg/m^3 Cr(VI) (95% CI: 0.12–0.31), while HBM4EU reported a GM of 0.3 μg Cr(VI)/m^3 outside RPE. Thus, in comparison with the Swedish and the pan-European study, the Danish Cr(VI) levels for bath plating were relatively high, being 3–5 fold higher as compared to the other contemporary European studies.

4.2. Exposure biomarkers

For all biomarkers of Cr(VI) exposure, bath platers had highly increased levels compared to controls, whereas welders represented in the category manufacturing and processing of metal products did not differ from the controls for any of the biomarkers of exposure.

4.2.1. Urinary Cr

Kristiansen et al. (1997) reported a reference interval for urinary Cr in the general population in Denmark to be a mean ± standard deviation of 5.2 nmol/L ± 4.1 nmol/L corresponding to 0.27 μg/L ± 0.21 μg/L (Kristiansen et al., 1997). Median values were 4.2 nmol/L corresponding to 0.22 μg/L with a 95% CI of 0.20–1.35 μg/L. In the present study, the within company controls had a mean urinary Cr of 0.78 μg/L and a median of 0.4 μg/L Cr. These values are higher than the reference values and higher than the outwith company controls in the Swedish SafeChrom study, where median post-shift urinary Cr values of 0.11 μg/L were reported (Jiang et al., 2024). This probably reflects that the within company controls in the current study were exposed to Cr to some extent. This is in agreement with the findings from the large European HBM4EU study (Santonen et al., 2022; Viegas et al., 2022), who reported higher urinary Cr for within company controls than for outwith company controls. HBM4EU reported urinary Cr for within company controls with median value of 0.3 μg Cr/g Crea, which is similar to the median urinary Cr for within company controls in the present study of 0.31 μg Cr/g Crea. In comparison, the HBM4EU outwith company controls had median urinary Cr of 0.1 μg Cr/g Crea, which is in line with the SafeChrom study outwith controls (median urinary Cr: 0.10 μg Cr/g Crea). Taken together, this suggests that the within company controls in the present study have increased urinary Cr as compared to Danish reference values and as compared to outwith company controls from other studies.

Occupational exposure to Cr(VI) via Cr in urine has been assessed in the two previously mentioned Danish studies of welders. The reference persons were metalworkers and electricians. In Bonde and Christensen (1991), MMA/stainless steel welders had median urinary Cr of 0.635 μg Cr/g Crea, TIG/stainless steel welders a median urinary Cr of 0.95 μg Cr/g Crea and mild-steel welders a urinary Cr of 0.60 μg Cr/g Crea (Bonde and Christensen, 1991). Metalworkers and electricians had median urinary Cr levels of 0.34 and 0.32 μg Cr/g Crea, respectively (Bonde and Christensen, 1991). In the study by Knudsen et al. (1992), the average post-shift mean Cr concentrations in urine from exposed stainless steel welders varied from 2.38 to 7.85 μmol/mol creatinine or 0.78–2.16 μg Cr/g Crea. The reference group had a mean urinary Cr of 0.37 ± 0.37 μg Cr/g Crea. In comparison, within company controls in the present study have very similar median urinary Cr (0.31 μg Cr/g Crea) as the reference persons in the 30-year-old studies by Bonde (Bonde and Christensen, 1991). The mean urinary Cr for within company controls in the present (0.62 μg Cr/g Crea) is higher than the mean urinary Cr of 0.37 μg Cr/g Crea in Knudsen et al. (1992). The welders in the current study had a median urinary Cr level of 0.65 μg Cr/g Crea, which is similar to the urinary Cr levels reported by Bonde (1991) for MMA and mild steel welders. The welders in the current study had a mean urinary Cr level of 0.87 μg Cr/g Crea, which is similar to the urinary mean Cr for MIG welders but lower than the urinary mean Cr values for MMA + TIG and TIG welders in Knudsen et al. The urinary Cr levels in welders in the current study was similar to the levels reported by HBM4EU (post-shift urinary Cr: median value 0.7 μg Cr/g Crea and mean value: 1.1 μg Cr/g Crea). Conversely, urinary Cr for welders was lower in the Swedish SafeChrom study (median urinary Cr: 0.41 μg Cr/g Crea). However, the type of welders in the present study and the Swedish study differed as the Swedish study included TIG welding and the values may therefore not be directly comparable. In the bath plating companies, Cr(VI) exposed workers had median urinary Cr value (4.68 μg/L) that exceeds the French BOELs of 2.5 μg/L. Similarly, workers with the work task ‘process operation’ (i.e. performing bath plating) had a median urinary Cr value of 4.70 μg/g Crea. In comparison, bath platers in HBM4EU had median urinary Cr levels of 1.1 μg Cr/g Crea (Viegas et al., 2022), and in SafeChrom, workers at bath plating companies had urinary Cr levels of 0.56 μg Cr/g Crea (Jiang et al., 2024). Thus, the Danish bath plates have much higher urinary Cr levels as compared to the Swedish and the European studies.

4.2.2. Cr in RBC

In the current study, workers with occupational exposure to Cr(VI) had significantly higher Cr levels in RBCs as compared to within company controls. When subdividing by company type, only workers within bath plating had significantly increased RBC levels of Cr (VI).

In the Swedish SafeChrom study, workers at bath-plating companies had median RBC levels of Cr of 0.83 μg/L. Bath platers in HBM4EU (Ndaw et al., 2022) had higher RBC levels of Cr (median value 4.34 μg/L) as compared to 1.33 μg/L in the current study for both Cr(VI) exposed workers at bath plating companies and workers with the work task ‘process operation’.

4.3. Interviews, questionnaire and observations of OSH risk prevention

Triangulation regarding OSH risk prevention through the use of interviews, a questionnaire and systematic safety observations revealed consistent results in terms of use of PPE and technical assistive devices such as local exhaust ventilation systems, and the use of plastic balls and sheet or cardboard coverings to reduce aerosol formation in bath plating. The relatively high OSH compliance rate reflected by the systematic safety observation safety indexes may be due to heightened attention given to the various data collectors by the companies on the day of data collection, as data were mainly collected on announced visits by the research team on the same day for both biomonitoring, air sampling, interviews, questionnaires and safety observations.

A recent systematic review on occupational exposure to Cr(VI) provides evidence of the use of OSH risk prevention measures such as technical solutions, PPE, job-rotation and limiting lengths of shift-work (Verdonck et al., 2021). A study in nine European countries (HBM4EU project) concluded that use of PPE in bath plating and welding showed lower urinary Cr (Viegas et al., 2022). In addition, results from the recent SafeChrom study in Sweden with visual observations in 113 Cr (VI) exposed workers showed lower levels of both compliance to use of local exhaustion ventilation and proper use of respiratory protective equipment (Jiang et al., 2024). The authors of the study from Sweden concluded that local exhaustion ventilation had a greater preventive effect than respiratory protective equipment, and that the results suggest that the use of respiratory protective equipment is inadequate due to factors such as other exposure routes, irregular use or premature removal of respiratory protective equipment during exposure, as well as lack of fit test presumably leading to leaking from the mask when worn (Jiang et al., 2024). Qualitative and quantitative fit testing can contribute to more effective use of respiratory protective equipment (HSE, 2019), however results of the current study reinforce the need to focus on applying the upper levels of the hierarchy of controls with eliminating or substituting Cr, and through the use of more effective technical solutions (e.g. automation) in reducing occupational exposure to Cr(VI), as supplements to the use of organizational measures (e.g. job rotation), and worker related measures (e.g. instruction, training and use of personal protective equipment).

4.4. Cr(VI) exposure in welders

In the current study, welders were statistically significantly exposed to Cr(VI) when assessed by air measurement but not by measurement of the actual exposure in blood or urine. However, the reason for this may primarily be the modest statistical power caused by the limited number of welders (n = 7–9) and the use of within company controls who have low Cr(VI) exposure. Comparing our findings to the data from the 30-year-old Danish studies of welders, may suggest that the Cr(VI) exposure of welders may have decreased over time as Cr(VI) in air was lower in the current study as compared to (Bonde and Christensen, 1991; Knudsen et al., 1992). However, the urinary Cr levels for welders in the current study were not substantially different from the reported levels in older Danish studies, and quite similar to the levels reported in the HBM4EU study. Notably, lower urinary Cr level were reported in the Swedish SafeChrom study. Despite the lower urinary Cr levels, urinary Cr was correlated positively with welding in linear regression analyses in the SafeChrom study (Jiang et al., 2024). This, in turn, suggests that for the Swedish welders, the increased urinary Cr levels were likely caused by occupational exposure.

4.5. Cr(VI) exposure in bath platers

We consistently found indications that workers with possible Cr(VI) exposure in path plating companies and workers with the work task 'process operation' (i.e. bath plating) had increased Cr(VI) exposure in terms of Cr(VI) in air, urinary Cr and Cr levels in red blood cells. Urinary Cr exceeded the French BOELs of 2.5 μg/L, which corresponds to an OEL of 1 $\mu g/m^3$ for bath plating, even though only a few air measurements exceeded the Danish OEL assuming that the air measurement are representative for an 8-h working day. This may indirectly suggest that other routes of exposure may contribute to Cr(VI) exposure in addition to inhalation. Both the interviews, questionnaires and systematic safety observations suggested high OSH compliance - at least on the day of observation. However, the biomarkers of exposure reflecting recent exposure (urinary Cr) or exposure during the last 4 months (Cr in RBC) may point to variation in OSH compliance in general. Similar high Cr exposure of bath platers was found in HBM4EU (Viegas et al., 2022), who suggested that oral exposure by hand to mouth contact may contribute to Cr(VI) exposure. In addition, in the HBM4EU study, it was shown that automated bath plating significantly reduced urinary Cr (Viegas et al., 2022).

4.6. Micronuclei

Genotoxicity was assessed in terms of MNRET in the current study. We found that Cr(VI) exposed workers had increased levels of MNRET as compared to within company controls. Specifically, workers in bath plating companies and workers with the work task 'process operation' had increased levels of MNRET as compared to within company controls (median micronuclei frequencies 1.89, 1.89 and 1.06, respectively.). Taken together with the increased levels of biomarkers of Cr(VI) exposure, this suggests that the increased MNRET levels may be attributed to occupational exposure.

In the HBM4EU chromate study, several biomarkers of genotoxicity were studied including MNRET. In general, levels of biomarkers of genotoxicity were increased in Cr(VI) exposed workers as compared to outwith company controls but not as compared to within company controls (Tavares et al., 2022). Specifically, MNRET levels were increased for all Cr(VI) exposed as compared to outwith company controls, and MNRET for welders was increased as compared to both outwith and within company controls. Of note, MNRET was assessed for four countries (Belgium, Finland, Poland and Portugal) and outwith company controls were only available for Portugal and Finland.

Knudsen et al. (1992) also assessed biomarkers of genotoxicity and reported increased levels of chromosomal aberrations in stainless steel welders as compared to within company controls (Knudsen et al., 1992).

Our results agree with those previous studies reporting chromosome damage. A major genotoxic effect of Cr(VI) that contributes to carcinogenesis is the formation of DNA adducts, which can lead to DNA damage (Alur et al., 2024). Intracellular reduction of Cr(VI) results in the generation of Cr(III), which forms several types of Cr-DNA adducts. If not properly repaired, Cr-DNA adducts may generate mutations and DNA double-stranded breaks (Krawic and Zhitkovich, 2023), which can give rise to micronuclei and chromosome aberrations.

Whereas increased levels of micronuclei in peripheral blood lymphocytes have shown to be predictive of cancer risk in prospective studies (Bolognesi et al., 2021; Bonassi et al., 2007; Dhillon et al., 2021), analysis of MNRET is a recent tool in human biomonitoring studies (Costa et al., 2011) and has not yet been assessed for predictivity of cancer risk. Nevertheless, MNRET is a promising mutagenicity biomarker, especially as indicator of potential leukemogenic agents (Albertini and Kaden, 2020).

4.7. Study strengths and limitations

This study on occupational exposure to Cr(VI) is to our knowledge the first in Denmark in 30 years. We used the same study protocol and questionnaires as the Swedish SafeChrom study and the large HBM4EU study with some modifications. Cr exposure in air was quantified using accredited methods that were aligned with the Swedish SafeChrom study. Thus, air measurements were performed according to the US EPA and Cr(VI) content in inhalable dust was quantified by LC-ICP-MS technique according to US EPA. For Cr in urine and blood, the work was based on the analysis protocol from Jiang et al., 2024) with an adapted volume of urine sampled (250 μL), a similar dilution factor and the same alkaline solution for the dilution (Jiang et al., 2024). For matrix matching of the calibration standards, SERNORM Whole blood L-1, Lot: 2011920 and urine GEQUAS 64/2019 8 A were prepared in the same way as the samples and spiked with Cr. Inductively coupled plasma mass spectrometry (ICP-MS), as applied in this study, was one of the two methods that was evaluated as appropriate for the determination of chromium in urine and whole blood regarding occupational exposure levels by the HBM4EU quality assurance program (Nübler et al., 2022). The LOQs in this study (0.16 μg/L for urine and 0.60 μg/L for blood) were below or similar to the mean LOQs reported in the HBM4EU inter-laboratory comparison (0.27–0.42 μg/L for urine and 0.42–0.95 μg/L for whole blood, depending on test round). The selected reference materials in this study had concentrations close to the low-level control materials used in the inter-laboratory comparison and satisfying precision and trueness were obtained. Thus, the current study should be comparable to the SafeChrom and HBM4EU studies (Jiang et al., 2024; Santonen et al., 2022). Furthermore, the present study describes NACE and ISCO-codes for the study population, which adds to transparency, and facilitates replication and pooling of the data.

The current study has a number of limitations. The most important limitation is the limited number of participants mainly caused by Covid-19 lock-downs. Challenges in the recruiting of companies and employees were also highlighted by the HBM4EU study (Galea et al., 2021). Furthermore, we note that the companies who agreed to participate likely represent companies who have special interest in occupational health and safety, and thus, may not be representative for the entire field, potentially leading to underestimation of the true exposure and accompanying risks. In addition, we only included employees who were able to read and write in Danish, again potentially introducing bias in the representativeness of the study participants. Another limitation is that Cr(VI) was only measured once for each study participant. We only collected one urine sample per day instead of morning and end-of-shift samples for logistic reasons. Both the HBM4EU study and SafeChrom convincingly showed increased urinary Cr levels in Cr(VI)-exposed workers could be attributed to occupational exposure (Jiang et al., 2024; Santonen et al., 2022). The Cr(VI)-exposed group were all male, whereas there were 4 women among the controls. The study group also included present smokers, even though smoking is suggested to contribute to Cr in blood and urine as well as MNRET (Offer et al., 2005). Of note, smoking status did not influence urinary Cr in a study of reference levels in Danes (Kristiansen et al., 1997). Due to the limited statistical power, we did not adjust the analyses for age, sex and smoking status. Of note, these adjustments did not influence the results in the SafeChrom study (Jiang et al., 2024). As the air measurements for most of the participants were done on the same day as the biological sample collection they may differ from the exposure levels present 3 days prior to day of measurement, which is the time when the RETMN were formed. Furthermore, three of the air measurements at one of the bath plating companies were performed five months after the biological sampling. However, the day-to-day variation in the exposure from the baths is assumed to be small, as the main source of Cr(VI) exposure is the baths. Another limitation is that the Cr(VI) air exposure levels were only measured for 3 h thus not reflecting an 8 h working day. If the worker change work task during the day this may influence the average daily exposure level.

5. Conclusion

The SAM-Krom study shows that bath platers are highly exposed to Cr(VI) as suggested by relatively high urinary Cr levels, Cr levels in RBC and increased levels of micronuclei. The urinary Cr levels were high as compared to the BOEL corresponding to 1 μg/m^3, thus indirectly suggesting that additional exposure routes contribute to the exposure. For welders, no statistically significant increases as compared to within company controls were observed, however, the observed urinary Cr levels were similar to the levels observed in HBM4EU and higher than the levels observed for welders in SafeChrom. On the announced visits, a consistent high degree of compliance to OSH risk prevention measures was seen in bath plating for both technical devices (ventilation, plastic balls, coverings) and use of personal protective equipment, and to a lesser degree of compliance to OSH risk prevention measures in welding with stainless steel. However, the biomarkers of exposure reflecting recent exposure (urinary Cr) or exposure during the last 4 months (Cr in RBC) may point to variation in OSH compliance in general. This, in turn, may imply that a more consistent focus on OSH risk prevention measures, according to the upper levels of the hierarchy of controls (e.g. substituting Cr, automation), would reduce occupational exposure to Cr (VI).

CRediT authorship contribution statement

Anne Thoustrup Saber: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Marcus Levin:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Pete Kines:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kukka Aimonen:** Writing – review & editing, Resources, Investigation. **Lucas Givelet:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Christina Andersen:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Anja Julie Huusom:** Writing – review & editing, Investigation. **Tanja Carøe:** Writing – review & editing, Investigation. **Niels Erik Ebbehøj:** Writing – review & editing, Methodology, Conceptualization. **Frans Møller Christensen:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Zheshun Jiang:** Writing – review & editing. **Thomas Lundh:** Writing – review & editing. **Håkan Tinnerberg:** Writing – review & editing. **Maria Albin:** Writing – review & editing. **Malin Engfeldt:** Writing – review & editing. **Karin Broberg:** Writing – review & editing, Funding acquisition. **Julia Catalan:** Writing – review & editing, Methodology, Funding acquisition. **Katrin Loeschner:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Karsten Fuglsang:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization. **Ulla Vogel:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

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Appendix A. Supplementary data

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