applied sciences

MDPI

Article

Fluoride Exposure and the Effect of Tobacco Smoking on Urinary Fluoride Levels in Primary Aluminum Workers

Marián Schwarz [1], Jozef Salva [1,*], Miroslav Vanek [1], Oqil Rasulov [2] and Ivana Darmová [3]

[1] Department of Environmental Engineering, Faculty of Ecology and Environmental Sciences, Technical University in Zvolen, T. G. Masaryka 24, 960 01 Zvolen, Slovakia; schwarz@tuzvo.sk (M.S.); vanek@tuzvo.sk (M.V.)
[2] Department of Life Safety and Environmental Protection, Faculty of Innovation and Technologies, Tajik Technical University, Academic Rajabovho 10, Dushanbe 734042, Tajikistan; oqil.rasulov@ttu.tj
[3] Regional Authority of Public Health in Žiar nad Hronom, Cyrila a Metoda 357/23, 965 01 Žiar nad Hronom, Slovakia; darmova@ruvzzh.sk
* Correspondence: xsalvaj@tuzvo.sk; Tel.: +421-907-475-988

Abstract: Workers in primary aluminum smelter are exposed to fluoride from cryolite (Na_3AlF_6) used in the electrolysis process. Post-shift urinary fluoride is considered as an appropriate index for examination of fluoride exposure. The objective of the study was to investigate the exposure to fluoride in primary aluminum smelter in Žiar nad Hronom (Slovakia) during three consecutive two-year periods between 2012 and 2018. The relationship between fluoride exposure in the occupational environment, tobacco smoking, and pre- and post-shift urinary fluoride concentration was investigated in 76 male workers in the ages from 21 to 60 years. Workers were monitored by personal fluoride sampling equipment. Their urinary samples were collected prior to the start and at the end of an eight-hour shift. Fluoride content in urine samples was analyzed by potentiometric ion-selective electrode and expressed as weight ratio of fluoride content to creatinine. The Mean ± SD particulate fluoride concentration in occupational air was 0.966 ± 1.658 mg/m^3 and gas-phase fluoride concentration was 0.327 ± 0.809 mg/m^3. Mean urinary fluoride concentration of all workers was significantly higher ($p < 0.001$) after the eight-hour shift. Smokers tended to have a higher post-shift mean urinary fluoride concentration than non-smokers, but this difference was not statistically significant ($p = 0.11$). The difference between these two groups of workers was also not statistically significant ($p = 0.62$) before the shift. Therefore, according to results, smoking caused no statistically significant difference in urinary fluoride levels between the group of smokers and group of non-smokers in primary aluminum workers.

Keywords: exposure; fluoride; primary aluminum; smoking; urinary fluoride

Citation: Schwarz, M.; Salva, J.; Vanek, M.; Rasulov, O.; Darmová, I. Fluoride Exposure and the Effect of Tobacco Smoking on Urinary Fluoride Levels in Primary Aluminum Workers. *Appl. Sci.* **2021**, *11*, 156. https://dx.doi.org/10.3390/app11010156

Received: 13 November 2020
Accepted: 23 December 2020
Published: 26 December 2020

1. Introduction

Primary aluminum production is one of the most important industries in the world with a total global production of 5438 thousand metric tonnes [1]. The mechanism of the primary aluminum production is electrolytic reduction (Hall–Héroult process) of aluminum oxide–alumina (Al_2O_3) dissolved in a molten fluoride electrolyte consisting of cryolite (Na_3AlF_6) at a temperature of about 960 °C. Electrolytic reduction is a continuous process inside steel container cells coated with carbon. It gives pure liquid aluminum metal at the negative carbon electrode (cathode) and carbon dioxide (CO_2) at the positive carbon electrode (anode) [2]. The anode slowly reacts under intense heat, carbon dioxide, and other gaseous emissions such as polycyclic aromatic hydrocarbons (PAH) and hydrogen fluoride (HF). Cell lines are situated in the potroom. Most current aluminum smelters use pre-bake anode technologies and the older plants use the Søderberg process [2,3].

The purpose of the fluoride components in the electrolyte is to lower the melting point to operate the cells at a lower temperature. Aluminum fluoride (AlF_3) neutralizes the sodium oxide (Na_2O), which is an impurity in the alumina feed. Fluoride emissions increase as the excess AlF_3 in the molten bath is increased [2]. According to a summary risk assessment report, 99.6% of all AlF_3 production is used by the aluminum industry [4].

In general, the gastrointestinal tract is the major route of fluoride uptake; however, in the occupational environment, fluoride is released into the air from industrial processes and workers are exposed to these substances by inhalation in both gaseous and particulate forms [5]. Emissions of fluoride dust (NaF, AlF_3, and unused cryolite) and fumes (PFCs and HF) dispersed into the occupational environment pose occupational health hazards [6].

Fluoride contamination is common at industrial sites, especially aluminum factories (smelters). The occupational health control of primary aluminum workers being exposed to fluoride focuses on the measurement of the occupational environment exposure and biological monitoring of fluoride concentration in urine. Fluoride metabolism in humans is well understood and according to Villa et al. [7], urinary fluoride excretion is the most important metabolic pathway for fluoride elimination from the body. The human body excretes rapidly about 50% absorbed amount of fluoride in urine (smaller amounts in feces, sweat and saliva) with a biological half-life of 2–9 h. The rest fluoride is deposited in the skeletal system and it is very slowly eliminated [8].

Post-shift urinary fluoride is considered as an appropriate index for surveillance of exposure to fluoride in aluminum smelter [9]. On the other hand, according to Aylward et al. [10], urinary flow and creatinine excretion are strongly individual and may vary from person to person. Further, factors like age, dose, renal impairment, composition of diet, and genetics can modify fluoride metabolism and alter the intake and excretion balance [11]. Secondary intake of fluoride in foods is also an important source even in non-contaminated areas. This can occur through consumption of tea and mineral water, by tobacco smoking and, in the pediatric population, also from toothpaste [12]. Cigarette smoking and tea consumption increase urinary fluoride, both separately and when combined [13]. In related research we have not found a detailed study that considered the effect of tobacco smoking on the fluoride content in the urine of primary aluminum workers. Generally, there is lack of information on the effect of tobacco smoking on fluoride levels in humans [13–15]. According to the scoping review by Idowu et al. [11], only a small proportion of previous studies investigated the association between fluoride intake and excretion. The research in this area should focus on detailed fluoride exposure and excretion sampling technique, and clearly define the relationship between intake and urinary excretion of fluoride.

The objectives of the present study were to measure the occupational exposure to fluoride in primary aluminum workers in the same smelter repeatedly over several years and to verify whether worker exposure to fluoride in the occupational environment is crucial for the absorption of fluorides and subsequent excretion in the urine. To examine the impact of fluoride exposure, the pre- and post-shift urinary fluoride concentrations were determined. The study also investigated the impact of tobacco smoking as the confounding factor of fluoride exposure. The research procedure and methodology were conducted in accordance with the requirements of the legislation of the Slovak republic [16], for the assessment of occupational environment. Thereby, the partial objective of the investigation was to verify whether this monitoring of fluoride exposure is representative.

2. Materials and Methods

2.1. Ethical Approval

All research procedures were approved prior the commencement of the study by the Ethics Committee of the Technical University in Zvolen, Slovakia.

2.2. Study Site

The study was conducted in primary aluminum production in Žiar nad Hronom, Slovakia. Production of primary aluminum has a strong tradition in Slovakia. The aluminum smelter in Žiar nad Hronom, Slovakia, started to operate in 1953. Since the start-up of production until the shutdown of the last Søderberg cell in 1996, approximately

2,000,000 tonnes of aluminum were produced in this factory. Alumina, as a base material for the production of primary aluminum, was produced from 1957 from purchased bauxite. This technology, in view of the environment and occupational conditions, was unsuitable. Due to anode paste baking and insufficient exhaustion of fumes to a treatment facility, enormous volumes of tar and fluorine were emitted into the environment. Between 1992 and 1995, there was a complete replacement of electrolysis technology; the Søderberg cells were replaced by a pre-bake anode system. Pre-bake technology significantly reduced the fugitive emission in the occupational environment due to its hermetic construction. During the research period, the number of cells in operation increased from 175 to the current 226. Each of the cells is connected to the suction and cleaning system, while during anode replacement, the suction capacity is increased. There are also organizational measures in place that limit the current number of open cells at the same time.

In Žiar nad Hronom, fluoride intake by ambient air was significant until 1992 when 10–30% of all samples exceeded the highest limit value for fluoride in air (5.0 mg/m^3). Between 1992 and 1995, classic Søderberg cells were gradually replaced with new technology and the concentration of fluoride in ambient air decreased to <1.0 $\mu g/m^3$ [12].

It should be noted that the concentrations of fluoride in stack emissions from smelter met the legislative requirements represented by emission limits. The emission limit for organized waste air exhausts in Slovak legislation has been determined only for HF at the level of 2.0 mg/m^3.

Further modernization of technological equipment and tightening of requirements for operational practice and emission limits (according to Best Available Techniques) continued to reduce the amount of fluoride emitted from the primary aluminum production plant in Žiar nad Hronom in following years.

2.3. Study Participants

In total, 76 male workers ranging in the ages from 21 to 60 years in an aluminum smelter were evaluated by spot urine samples. The age of each individual was not recorded for the purposes of the study. Data on the average age of the whole group for the particular year are available from the employer as follows: 2012: 46.4 years (n = 20); 2014: 37.5 years (n = 20); 2016: 39.5 years (n = 19); 2018: 41.4 years (n = 17).

Samples were collected prior to the start of an eight-hour shift and again at the end of the shift. Sampling occurred during three consecutive two-year periods between 2012 and 2018. Each study participant had been performing his working activity for more than a year. Work positions located directly in the potroom were investigated; in this area, fluoride exposure during the shift is at the highest level. At the same time, chemical determination and analysis of fluoride content in occupational air was carried out.

Samples of urine were analyzed for fluoride; results were expressed as weight ratio of fluoride content to creatinine (urinary fluoride). Workers' smoking habits were obtained using a questionnaire. The distribution of primary aluminum production workers who participated in the study was relatively uniform over the duration of the study. In total, 12 work positions were monitored (see Table 1). The worker position was not recorded in one instance. Of the 76 study participants, 35 were regular smokers (>10 cigarettes per day) and 39 were non-smokers. In two cases, the participants did not comment on the questionnaire about their smoking habits. Due to shift work, employee fluctuation, and the two-year study period (in accordance with the requirements of the legislation [16]), it was not possible to examine real working conditions every year for the same participants.

Table 1. Monitored work positions in primary aluminum smelter.

Number of Participants	Work Position
$n = 7$	Crane operator
$n = 10$	Forklift operator
$n = 2$	Anode transport
$n = 1$	Casting of alloys
$n = 8$	Preparation of smelting in the foundry, transport of dross
$n = 11$	Cell operator
$n = 19$	Anode replacement
$n = 7$	Pumping wagon operator
$n = 1$	Primary refining
$n = 3$	Cell start
$n = 1$	Measurement technician
$n = 5$	Pumping and transport of liquid metal

For more detailed information on monitored participants in each year of the research, see Appendix A.

The majority (84%) of workers who participated in the study came either directly from the city of Žiar nad Hronom, Slovakia, or from the surrounding area (<30 km). Thus, it is possible that participants also had environmental exposure to fluoride through potable water, ambient air, or by edibles. The content of fluoride in ambient air was discussed in Section 2.2 and its impact as confounding factor was considered negligible in this research. Fluoride intake by potable water is minimal in Žiar nad Hronom because the city and its surroundings are supplied by bulk potable water from distant non-contaminated sources where the quality of potable water must meet the hygienic standards (limit value 1.50 mg fluoride per liter). This water is also used as drinking water by employees of the smelter, thus also by all study participants. According to the latest comprehensive study of fluoride occurrence in Žiar nad Hronom, fluoride content from the bulk potable water supply and from private potable water wells from 1986 to 1997 did not exceed the highest limit value for fluoride for potable water; the average values in individual localities ranged from <0.15 to 1.45 mg/L. It should be noted that a major change in electrolysis technology occurred in 1992–1995, when a new electrolysis was built; the old blocks of the plant were gradually disconnected [12].

2.4. Air Sampling and Analyses

Sampling of fluoride in the occupational air was performed in accordance with the NIOSH method, using nitrocellulose membrane filters with a pore size of 0.45 μm for particulate fluoride sampling [17]. Filters were placed inside plastic cassettes together with MILLIPORE cellulose filters impregnated with sodium formate for gaseous phase sampling. The sampling apparatus was supplemented by a calibrated pump and worn by each participant during the shift. After sampling, the exposed filters were dried in a desiccator. Subsequently, nitrocellulose membrane filters were decomposed in sodium hydroxide solution and the solid fluoride content was analyzed by potentiometric ion-selective electrode on an ORION 4 STAR analytical instrument. Gas-phase fluoride captured on impregnated cellulose filters were analyzed using the same procedure. The analytical method was controlled by certified reference material ASTASOL. The determination of the particulate concentration was performed by the gravimetric method. The weight of the collected fluoride content was determined from the difference in weight of the filters before and after the measurement. Particulate concentration was calculated by dividing the weight of particulate matter collected on the filter by the pumped air volume recalculated on standard condition ($T = 293.15$ K, $p = 101.325$ kPa).

Workers in the potroom wore personal respiratory protection equipped with organic vapors/acid gas cartridges and dust prefilter.

2.5. Urine Sampling and Analyses

The urine samples of each participant were taken before and after an eight-hour shift. The urine was collected in sterile plastic containers. The analysis of fluoride in urine was performed by the NIOSH method [18]. In the analysis, the urine was diluted with an auxiliary solution of Total Ionic Strength Adjustment Buffer (TISAB) to adjust the pH and ionic strength of the solution to release fluoride ions from possible aluminum and ferric complexes. TISAB was prepared by diluting 57 mL of glacial acetic acid, 58 g sodium chloride, and 4 g trans-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid monohydrate in 1000 mL water and subsequently adjusted to pH 5.2–5.4 with sodium hydroxide, 20% (w/v). A sample of 25 mL of urine was added to an equal volume of auxiliary solution (TISAB). Subsequently, magnetic stirring was switched on. Two electrodes were used for the determination: ion selective fluoride electrode and a calomel electrode, both immersed in stirring solution. It is necessary to wait until the potential stabilizes while stirring (approximately 10 min). The fluoride concentration in urine was acquired from the calibration curve.

Picric acid reacts with creatinine in an alkaline medium to produce an orange-red color, which is measured photometrically. The determination of creatinine concentration in the urine is carried out in order to suppress errors in the calculation of the foreign substance concentration due to diuresis. Creatinine is excreted from an organism at a relatively constant rate, so the data calculated for creatinine are more accurate than the concentration of native urine [19]. The result is expressed as the amount of fluoride in mg corresponding to 1 g of creatinine. The determination of creatinine in urine was performed by NIOSH methods [20,21].

2.6. Statistical Analysis

Data were analyzed by STATISTICA (StatSoft Inc., Tulsa, OK, USA, ver. 12). Statistical significance was set at $p < 0.05$. Normality of the data was evaluated by Shapiro–Wilk (S-W) test and Kolmogorov–Smirnov (K-S) test. The results of these tests rejected normality of all variables ($p < 0.05$).

3. Results and Discussion

Table 2 shows the descriptive statistics for all study participants (for each study participant´s data, see Appendix A), group of smokers and non-smokers including measured data of air samples and urinary fluoride samples.

Among the monitored work positions in Table 1 there were no statistically significant differences ($p > 0.05$) for measured fluoride concentrations (particulate and gas-phase fluoride) in the occupational air. Within the total fluoride exposure, workers are the most exposed to the particulate fluoride emissions. The highest total (particulate + gas phase) fluoride concentration (12.120 mg/m^3) was registered in anode replacement work position. Our findings are consistent with the assumption that during anode replacement the worker is temporarily exposed to the open environment of the steel container and despite the increased efficiency of the air conditioning, the highest rate of fluoride exposure occurs in this operation. By contrast, the lowest concentration (0.018 mg/m^3) was found for the crane operator who is relatively isolated from direct exposure in the crane cabin.

A similar conclusion was reached by Ehrnebo and Ekstrand [22] who investigated 41 workers in an aluminum plant in Sweden during an eight-hour work shift; the mean total fluoride exposure in this study was 0.91 mg/m^3, of which 34% was HF. For comparison, Seixas et al. [9] found mean particulate fluoride and gas-phase fluoride (HF) concentrations were 4.1 and 0.7 mg/m^3 when they evaluated the relationship between particulate fluoride exposure and urinary excretion in an aluminum smelter.

Table 2. Mean fluoride concentration, standard deviation, and maximum and minimum values in occupational air and in urine samples.

Group	Statistical Indicator	Particulate Fluoride Concentration [mg/m^3]	Gas-Phase Fluoride (HF) Concentration [mg/m^3]	Pre-Shift Urinary Fluoride [mg/g Creatinine]	Post-Shift Urinary Fluoride [mg/g Creatinine]
All participants	Mean ± SD	0.966 ± 1.658	0.327 ± 0.809	0.455 ± 0.353	0.957 ± 0.686
	Median	0.285	0.112	0.385	0.880
	Range	0.009–10.290	0–6.210	0.100–2.420	0.180–3.730
		n = 74 [1]	n = 74 [1]	n = 76	n = 76
Smokers	Mean ± SD	0.992 ± 1.427	0.438 ± 1.143	0.441 ± 0.338	1.050 ± 0.708
	Median	0.370	0.104	0.360	0.890
	Range	0.009–5.910	0.009–6.210	0.120–2.090	0.280–3.730
		n = 33	n = 33	n = 35	n = 35
Non-smokers	Mean ± SD	0.985 ± 1.879	0.245 ± 0.368	0.469 ± 0.379	0.859 ± 0.677
	Median	0.240	0.140	0.430	0.770
	Range	0.009–10.290	0–1.720	0.100–2.420	0.180–3.370
		n = 39	n = 39	n = 39	n = 39

[1] In two cases, due to a sampling equipment failure, no values were recorded.

Contrary to determined distribution of particulate and gas-phase fluoride in the occupational environment of investigated potroom, the Risk assessment report [4] concluded that the average fluoride emission of a primary aluminum smelter within the EU consists of 53% HF and 47% particulate fluoride. This may be explained due to the composition of study participants. The majority of workers were exposed to emissions during anode replacement in cells, including the particulates during crust cleaning of the anode cavity before installing new anodes. Gas-phase fluoride is formed in electrolysis process directly in the cell and it is emitted especially during anode replacement process; however, particulates are formed from handling by application of cryolite powder in the molten bath mixture and when crust is disrupted. Crust is a solid matter transformed from anode covering material by heat [23].

The Kruskal–Wallis test (K-W test) showed that there was no statistically significant difference (p = 0.57) in particulate fluoride concentrations in the monitored years of the research. However, gas-phase concentrations were statistically significantly different (p = 0.003). As can be seen in Figure 1, this was due to increased gas-phase fluoride concentrations in 2014 (an approximately five times higher mean value compared to other years).

Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) of fluorides (as F) at a value of 2.5 mg/m^3 (as time-weighted average) was exceeded (2.67–10.29) in 10 workers (see Appendix A) [24]. Compared to the general population, inhalation of fluoride present in ambient air does not contribute more than 0.01 mg per day to the total fluoride intake [25]. Since 1996, long-term average annual concentrations of fluorides in ambient air are <1 µg/m^3 in the district of Žiar nad Hronom [12,26].

Figure 2 shows a comparison of mean ± SE urinary fluoride concentrations during the research period. No statistically significant difference (p > 0.05) was found for either pre- or post-shift urinary fluoride over the monitored years.

The most effective way to examine fluoride exposure provides the relation between the concentration in the air and the quantities excreted in the urine at the end of the shift [27].

The results of correlation analysis between fluoride exposure in the occupational environment (i.e., by inhalation) and corresponding urinary fluoride levels in monitored participants are presented in Table 3 and Figure 3.

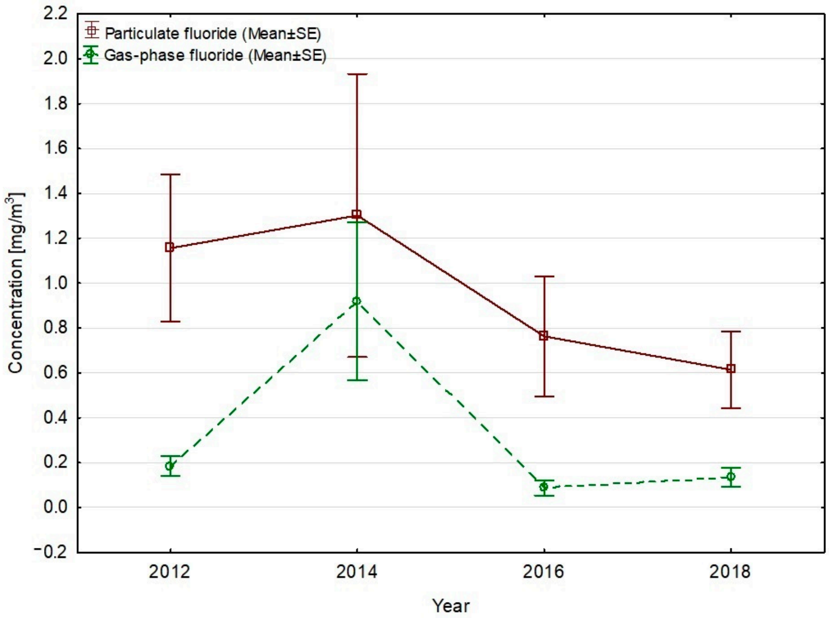

Figure 1. Comparison of the determined concentrations of fluoride in the occupational environment during the research period.

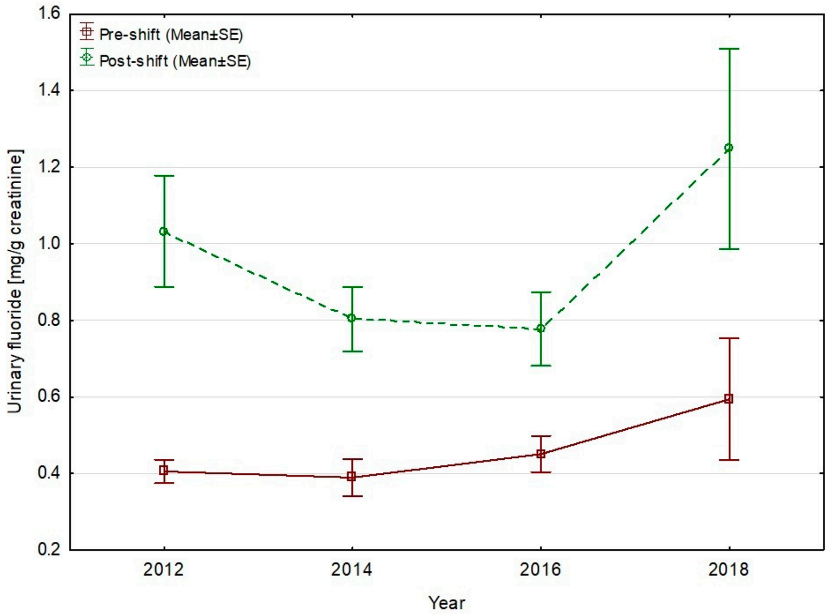

Figure 2. Comparison of urinary fluoride concentrations during the research period.

Table 3. Relationship between fluoride concentrations in the occupational environment and post-shift urinary levels (Pearson correlation test result coefficient R, and *p* value).

Parameter	Parameter
	Post-Shift Urinary Fluoride [mg/g creatinine]
Particulate fluoride concentration [mg/m^3]	0.039 (p = 0.74)
Gas-phase fluoride concentration [mg/m^3]	0.005 (p = 0.96)
Total fluoride concentration [mg/m^3]	0.029 (p = 0.81)

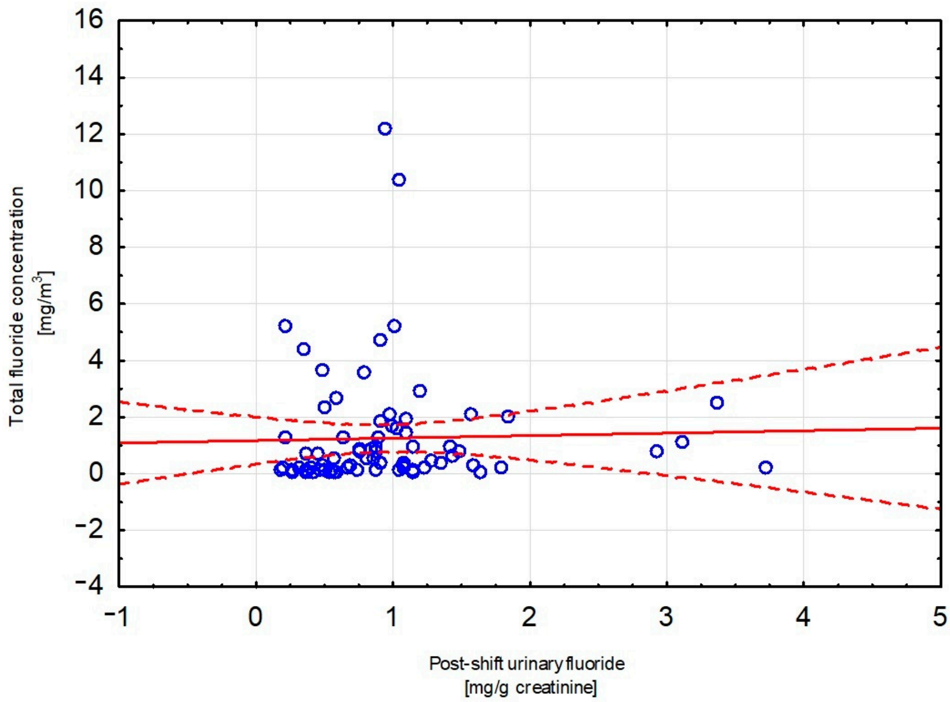

Figure 3. Correlation graph between total (particulate + gas-phase) fluoride concentrations in the occupational environment and post-shift urinary levels.

No significant correlation between fluoride concentrations in the occupational environment and in urine corrected to creatinine was found in the present study. Pierre et al. [27] reported an association between fluoride concentration of 2.5 mg/m^3 (OSHA PEL) in an occupational environment and post-shift urinary fluoride concentration of 6.4 mg/g creatinine, and a peak value of 7.4 mg/g creatinine, respectively. As mentioned above, our results showed OSHA PEL exceeded in 10 workers. In contrast with Pierre et al. [27], post-shift urinary fluoride levels in this workers reached only 0.2–1.2 mg/g creatinine and peak post-shift urinary fluoride 3.73 mg/g creatinine corresponds to 0.16 mg/m^3 of total fluoride concentration in the occupational environment. More than 50% exceeded samples above OSHA PEL were associated with anode handling working position (i.e., anode replacement, cell operator). Post-shift urinary fluoride levels in these workers proved that personal respiratory protection used during high exposure periods while working over open cells was effective. A similar conclusion was reached by Seixas et al. [9] who ascribed the lack of relationship between particulate fluoride exposure and the post-shift urinary fluoride by the effective use of respiratory protection in carbon setters (anode replacement).

Kono et al. [28] found a linear relationship between HF concentration (>5 ppm) in the occupational environment and mean urinary fluoride levels in electronics industry workers. The wide variation of fluoride levels in urine has been ascribed to the consumption of tea and seafood (water intake was not considered because only <1% of the water supply is fluoridated in Japan). In agreement with Kono et al. [28], we also rejected the fluoride intake by potable water in Žiar nad Hronom which met hygienic standards. In the occupational environment, fluoride intake via inhalation can reach 16.8 mg per day; nevertheless, daily intake of fluoride by optimally fluoridated potable water is about 1.4–2.4 mg [8]. During our research, participants were asked to avoid tea consumption and use of toothpastes enriched with fluoride for at least 48 h prior to urine collection; however, an apparent limitation of this procedure is its verification. Waugh et al. [29] and Koç et al. [13] concluded that tea is a significant source of fluoride intake. A major source of limitation in this study is fluoride intake effected by the composition of diet. With low water fluoride, the urinary fluoride concentration is much more influenced by eating habits [27]. According to Whitford [30], vegetarian diet leads to more alkaline urine and in addition increases the urinary fluoride excretion. Assessment of fluoride intake by food is quite challenging due to exposure to multiple dietary sources and supplements. Some authors in other studies used diet standardization to reduce this limitation, however, it is quite difficult to conduct [7].

Urinary fluoride concentrations of 0.8–1.2 mg/L are regarded as indicating optimal exposure to fluoride in population [31]. Figure 4 compares pre- and post-shift urinary fluoride concentrations for all study participants.

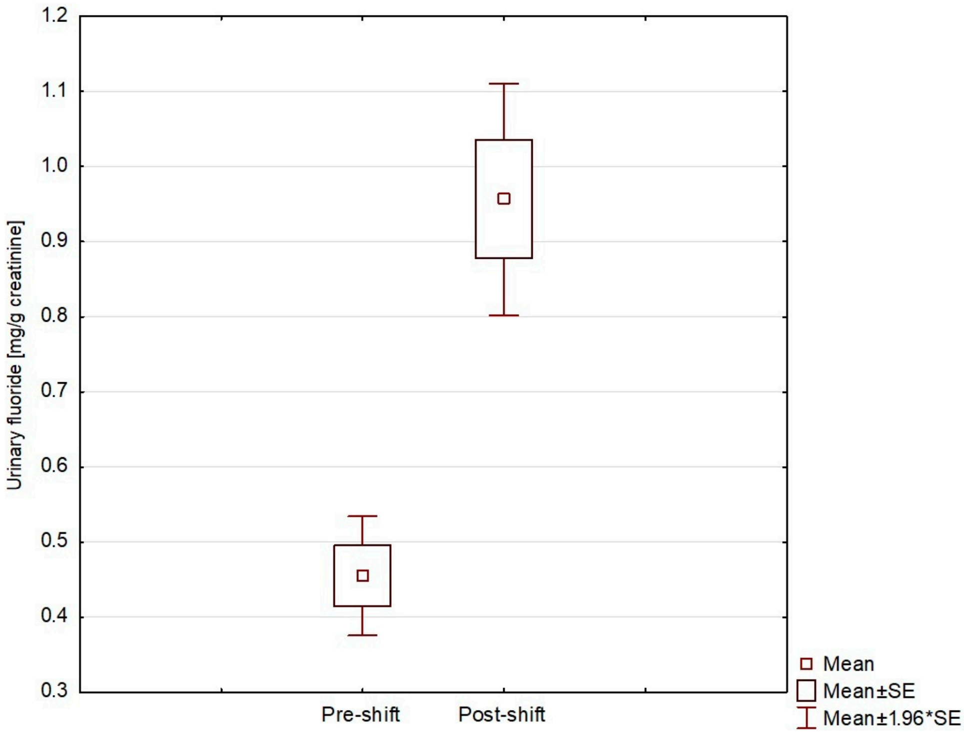

Figure 4. Comparison of pre-shift and post-shift urinary fluoride concentration for all participants.

According to Wilcoxon signed-rank test, mean post-shift urinary fluoride concentration was significantly ($p < 0.001$) higher compared to mean pre-shift urinary fluoride concentration for all investigated participants. From this, it can be concluded that there is an increase in fluoride concentration in primary aluminum workers during their work performance, although the relationship between occupational exposure and urinary fluoride levels was not confirmed. Overall, these findings are in accordance with previous findings reported by Rees et al. [32] who found significant ($p < 0.05$) difference between mean pre- and post-shift concentrations in workers exposed above OSHA PEL (2.5 mg/m^3) to calcium fluoride (CaF_2), and a weak correlation between intensity of occupational exposure to calcium fluoride and post-shift urinary fluoride concentration. Mean fluoride exposure in the dustiest environment was 24.3 mg/m^3 (40.6% respirable fraction) which is approximately 10 times OSHA PEL. Only one urinary concentration exceeded the recommended limit value of 7 mg fluoride per liter. It is important to highlight the fact that potroom of aluminum smelter is a high-temperature workplace; therefore, the relating of the urinary excretion of fluoride to g-creatinine is necessary. According to the World Health Organization (WHO) [31], the mean 24-h urinary creatinine value is 15 mg/kg of body weight per day. Several previous studies have not reported creatinine correction. Zohouri et al. [33] reported the mean urinary fluoride concentration of 1.49 (±0.63) mg/g creatinine in children.

The biological exposure index (BEI) for fluoride exposure is 3 mg/g creatinine prior to shift and 10 mg/g creatinine at end of shift [34]. The BEI were not exceeded in the monitored spot urine samples. We found that maximum pre- and post-shift urinary fluoride concentrations were 2.42 mg/g creatinine and 3.73 mg/g creatinine.

In agreement with WHO [31], the time of urine collection was approximately equal during the whole study period, which is important for correct interpretation of the results, because urine that has accumulated in the body over a shorter time period may manifest a short-lived peak of the fluoride concentration. The findings of the present study demonstrate variations in urinary fluoride levels within participants. We assume that improper use of a personal respiratory protection might contribute to these differences.

According to Seixas et al. [9], mean urinary fluoride concentrations were 1.3 and 3.0 mg/g creatinine in pre-shift and post-shift urine samples. The results of our study showed markedly lower mean concentrations of particulate fluoride and HF in the occupational environment. Our results also showed lower pre- and post-shift urinary fluoride concentrations. Research by Seixas et al. [9] was performed on a sample of 32 workers in a shorter period (days 1 and 3 of a three-day workweek), making it more sensitive to local and short-term increases in fluoride concentration in the occupational environment. On the other hand, our study provided information obtained from a longer time period and various operational situations that may have occurred in aluminum smelter.

A frequency distribution in Figure 5 shows that 89% of all study participants had urinary fluoride difference (post-shift minus pre-shift concentration) below 1 mg/g creatinine, with the maximum value at 3.38 mg/g creatinine and the minimum was at a negative value of −0.82 mg/g creatinine (a negative result of urinary fluoride difference was found in nine workers). According to Lauwerys et al. [35], the difference between post- and pre-shift urinary fluoride concentration should not exceed 3 to 4 mg/g creatinine, respectively, pre-shift values should be <3 mg/g creatinine.

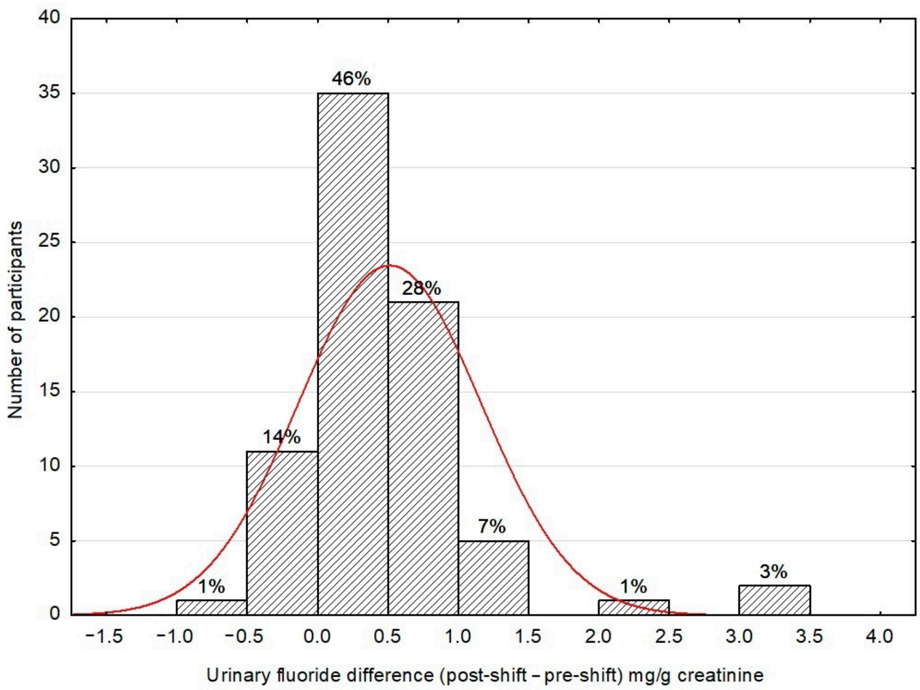

Figure 5. Frequency distribution of urinary fluoride difference for all study participants.

Tobacco smoking was the main confounding factor of fluoride exposure we decided to investigate. In total, 35 study participants were regular smokers consuming at least 10 cigarettes per day. Other 39 participants declared they were strict non-smokers and they were not aware of the regular secondhand smoke exposure. There was a separate smoking area in smelter. Workers had the right to one uninterrupted 30-min rest break during their working day, if they worked 8 h per day. Majority of smokers took this break also as smoking break. Further, each of the smokers confirmed that they smoked at least one cigarette before their shift.

Figure 6 shows a comparison of mean pre- and post-shift urinary fluoride concentrations according to smoking habits of investigated primary aluminum workers. The results in the present study showed no statistically significant difference of the urinary fluoride level among group of smokers and non-smokers before the shift ($p = 0.62$) and after the shift ($p = 0.11$) by K-W test. This suggests that under the given research conditions, tobacco smoking does not affect the fluoride content of workers in primary aluminum smelter. It remains unclear why non-smokers show higher or equal pre-shift mean urinary fluoride concentration (0.469 ± 0.379 mg/g creatinine) than smokers (0.441 ± 0.338 mg/g creatinine). We assume that pre-shift samples reflected the worker´s total burden of fluoride and although smokers consumed a cigarette before shift, this was not reflected despite to rapid kinetics and the biological half-life of fluoride in human body. From the difference between pre- and post-shift urinary fluoride values, a decreasing trend of urinary fluoride concentration in smokers was found ($r = -0.133$, $p = 0.447$). The group of non-smokers was characterized by a slightly increasing trend ($r = 0.03$, $p = 0.868$).

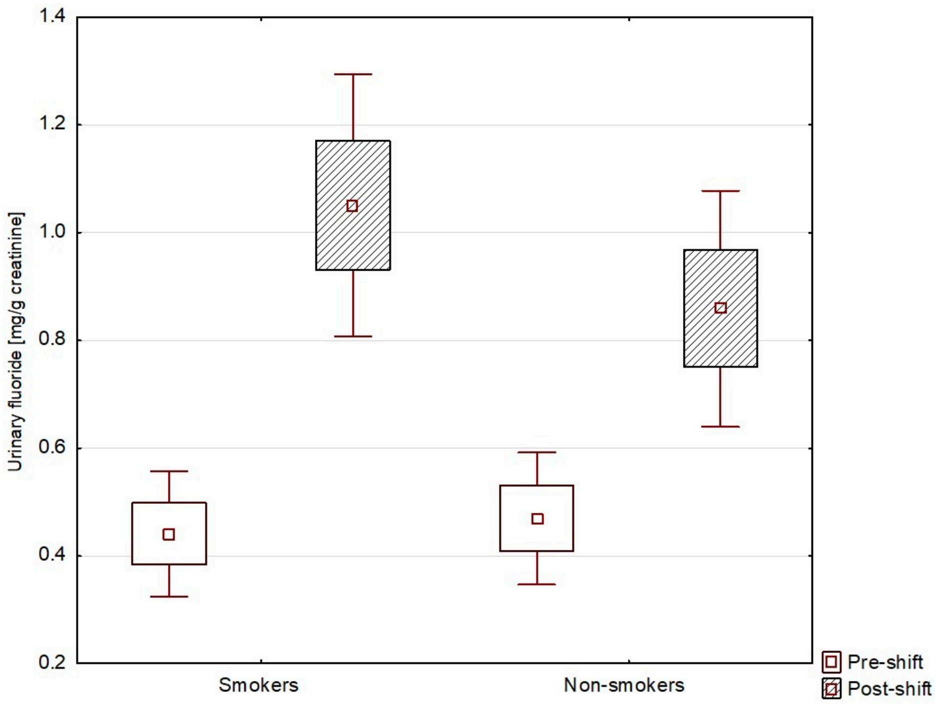

Figure 6. Comparison of pre- and post-shift urinary fluoride concentration according to smoking habits (boxplot: mean ± SE; whisker: mean ± 0.95 conf. interval).

The research on the interaction between tobacco smoking and fluoride absorption and, subsequently, excretion is limited. It is estimated that heavy cigarette smoking could daily contribute about 0.01 mg of fluoride intake per kg of body weight [36]. Laisalmi et al. [37] reported the relationship between smoking and inorganic fluoride levels due to anesthesia with enflurane (2-chloro-1,1,2,-trifluoroethyl-difluoromethyl ether). In contrast with the present study, they observed that the serum fluoride concentrations were significantly different between group of smokers and non-smokers. On the other hand, our results are in agreement with Radon et al. [38], who found no combined effect of smoking and occupational exposure in aluminum potroom workers exposed to hydrogen fluoride and inhalable dust below OSHA PEL. A similar conclusion also reached Tu et al. [39], who concluded that age, work history, smoking, and alcohol consumption did not affect the blood fluoride and urinary fluoride levels in 300 workers from an aluminum plant in China.

According to Koç et al. [13], urinary fluoride concentration is higher in smokers compared to non-smokers among 300 students at the University of Kafkas, Turkey. The present study found no significant differences between pre- and post-shift urinary fluoride levels in groups of smokers and non-smokers, although non-smokers had slightly higher levels of pre-shift urinary fluoride. This effect may be caused by other confounding variables, which we did not consider, such as drinking tea, dietary intake of fluoride, or metabolic disorders. Therefore, further research needs to be supplemented with data on excessive intake of fluoride.

4. Conclusions

The results of the present study showed a statistically significant increase of fluoride in the urine of primary aluminum workers during an eight-hour shift. The research procedure and methodology provide an effective indication of fluoride exposure in aluminum smelter in comparison with BEI. However, the source of fluoride intake in these workers was not clarified. The degree of correlation between occupational exposure to fluoride and urinary fluoride levels was ascribed to person respiratory protection. Future investigations are necessary to validate conclusions that can be drawn from this study. It will be important for future research to conduct repeated measurements for the same workers for a longer time period. A single spot urine sample from an individual may be affected by many strongly variable confounding factors. Collecting the post-shift urine samples after several working days would be appropriate and more representative. The study showed that tobacco smoking caused no statistically significant difference in urinary fluoride levels between group of smoker and non-smokers; however, further investigations are needed to confirm these results. Overall, further research should address these issues. We would like to focus on the assessment of fluoride intake from tea consumption, fluoride toothpastes, and dietary intake in primary aluminum workers.

Author Contributions: Conceptualization, M.S. and J.S.; methodology, M.S.; software, J.S.; validation, M.V., J.S., and O.R.; formal analysis, M.V.; investigation, M.S. and J.S.; resources, M.S. and I.D.; data curation, J.S.; writing—original draft preparation, J.S.; writing—review and editing, O.R.; visualization, J.S.; supervision, M.S.; project administration, M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: For this type of study a formal IRB approval is not required. Study involves data collected from individual subjects that cannot be identified directly or through identifiers linked to the subjects.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available within the article.

Acknowledgments: The paper is based on work performed under research contract KEGA 008TU Z-4/2019. The authors gratefully thank Slovalco a.s. and PRO CARE Žiar nad Hronom for their assistance and cooperation.

Conflicts of Interest: The authors declare that there is no conflict of interest.

Appendix A

Table A1. Data on study participants and their occupational exposure (particulate and gas-phase fluoride concentration detected by personal sampling), urinary fluoride biological monitoring (pre- and post-shift urinary fluoride), tobacco smoking habit, and work position.

Participant	Year	Particulate Fluoride Concentration [mg/m^3]	Gas-Phase Fluoride (HF) Concentration [mg/m^3]	Pre-Shift Urinary Fluoride [mg/g Creatinine]	Post-Shift Urinary Fluoride [mg/g Creatinine]	Tobacco Smoking	Work Position
1.	2012	1.30	0.14	0.54	1.11	smoker	anode replacement
2.	2012	2.04	0.46	0.28	3.37	non-smoker	anode replacement
3.	2012	0.39	0.15	0.43	0.57	non-smoker	crane operator
4.	2012	4.82	0.36	0.56	0.22	non-smoker	forklift operator

Table A1. *Cont.*

Participant	Year	Particulate Fluoride Concentration [mg/m^3]	Gas-Phase Fluoride (HF) Concentration [mg/m^3]	Pre-Shift Urinary Fluoride [mg/g Creatinine]	Post-Shift Urinary Fluoride [mg/g Creatinine]	Tobacco Smoking	Work Position
5.	2012	1.92	0.18	0.60	0.99	non-smoker	anode replacement
6.	2012	0.26	0.15	0.47	1.29	unknown	forklift operator
7.	2012	0.44	0.15	0.73	1.44	non-smoker	anode replacement
8.	2012	0.08	0.01	0.38	0.38	non-smoker	pumping wagon operator
9.	2012	0.03	0.04	0.25	1.05	smoker	casting of alloys
10.	2012	0.03	0.01	0.43	0.42	non-smoker	preparation of smelting in the foundry, transport of dross
11.	2012	0.04	0.01	0.37	1.16	unknown	preparation of smelting in the foundry, transport of dross
12.	2012	0.17	0.03	0.41	1.24	non-smoker	primary refining
13.	2012	0.10	0.02	0.36	0.56	smoker	pumping wagon operator
14.	2012	0.21	0.09	0.36	1.09	smoker	cell start
15.	2012	1.74	0.21	0.31	1.11	non-smoker	preparation of smelting in the foundry, transport of dross
16.	2012	3.26	0.25	0.28	0.80	non-smoker	anode replacement
17.	2012	0.63	0.23	0.46	0.85	non-smoker	cell operator
18.	2012	0.20	0.19	0.10	1.35	non-smoker	anode replacement
19.	2012	4.34	0.85	0.43	1.01	smoker	pumping wagon operator
20.	2012	1.12	0.14	0.36	0.65	non-smoker	cell operator
21.	2014	0.009	0.66	0.12	0.45	non-smoker	crane operator
22.	2014	10.29	0.10	0.74	1.05	non-smoker	crane operator
23.	2014	0.009	0.95	0.39	1.15	non-smoker	forklift operator
24.	2014	0.89	0.36	0.16	0.22	non-smoker	forklift operator
25.	2014	-	-	0.12	0.60	smoker	anode replacement
26.	2014	-	-	0.33	1.64	smoker	forklift operator
27.	2014	0.009	0.13	0.18	0.89	smoker	anode replacement
28.	2014	0.009	0.22	0.25	0.50	non-smoker	anode replacement
29.	2014	0.91	2.68	0.14	0.50	smoker	cell operator
30.	2014	0.009	0.14	0.12	0.21	non-smoker	anode transport
31.	2014	0.72	0.23	0.57	0.89	smoker	crane operator
32.	2014	0.53	0.38	0.84	1.42	smoker	crane operator
33.	2014	0.009	0.31	0.63	1.09	smoker	forklift operator
34.	2014	0.34	1.31	0.55	1.00	non-smoker	anode replacement
35.	2014	5.91	6.21	0.46	0.95	smoker	anode replacement
36.	2014	0.31	0.37	0.35	0.38	smoker	cell operator
37.	2014	0.51	0.009	0.57	0.87	non-smoker	cell operator
38.	2014	2.95	1.72	0.5	0.91	non-smoker	anode transport
39.	2014	0.009	0.74	0.43	0.76	smoker	pumping and transport of liquid metal
40.	2014	0.009	0.03	0.34	0.58	smoker	preparation of smelting in the foundry, transport of dross
41.	2016	0.15	0.03	0.35	0.33	smoker	crane operator
42.	2016	0.06	0.04	0.49	0.51	non-smoker	unknown
43.	2016	0.08	0.06	0.18	1.16	smoker	forklift operator
44.	2016	0.10	0.03	0.20	0.48	non-smoker	cell operator
45.	2016	4.36	0.04	0.33	0.35	smoker	anode replacement
46.	2016	0.80	0.04	0.82	0.77	non-smoker	cell operator
47.	2016	2.67	0.23	0.32	1.20	non-smoker	anode replacement
48.	2016	0.009	0.05	0.56	0.55	non-smoker	pumping wagon operator
49.	2016	0.22	0.009	0.27	0.70	smoker	pumping wagon operator
50.	2016	0.07	0.091	0.26	0.41	non-smoker	preparation of smelting in the foundry, transport of dross
51.	2016	0.71	0.082	0.50	1.50	smoker	cell operator
52.	2016	1.87	0.15	0.48	1.85	smoker	cell operator
53.	2016	2.02	0.65	0.85	0.60	smoker	forklift operator
54.	2016	0.009	0.009	0.75	0.43	non-smoker	crane operator
55.	2016	0.37	0.009	0.47	0.91	non-smoker	anode replacement
56.	2016	0.66	0.07	0.36	0.89	smoker	anode replacement
57.	2016	0.009	0.009	0.21	0.37	non-smoker	preparation of smelting in the foundry, transport of dross
58.	2016	0.19	0.03	0.54	0.68	smoker	pumping wagon operator
59.	2016	0.14	0.03	0.63	1.08	non-smoker	pumping wagon operator
60.	2018	0.07	0.06	0.23	0.74	smoker	anode replacement

Table A1. *Cont.*

Participant	Year	Particulate Fluoride Concentration [mg/m^3]	Gas-Phase Fluoride (HF) Concentration [mg/m^3]	Pre-Shift Urinary Fluoride [mg/g Creatinine]	Post-Shift Urinary Fluoride [mg/g Creatinine]	Tobacco Smoking	Work Position
61.	2018	0.11	0.00	0.20	0.18	non-smoker	forklift operator
62.	2018	2.24	0.07	0.51	0.51	smoker	anode replacement
63.	2018	0.24	0.04	2.42	1.60	non-smoker	cell start
64.	2018	1.92	0.17	0.19	1.57	smoker	cell operator
65.	2018	0.91	0.64	0.48	1.03	non-smoker	measurement technician
66.	2018	0.19	0.02	0.22	0.41	non-smoker	pumping and transport of liquid metal
67.	2018	0.12	0.04	0.35	3.73	smoker	pumping and transport of liquid metal
68.	2018	0.02	0.01	0.20	0.28	non-smoker	preparation of smelting in the foundry, transport of dross
69.	2018	0.37	0.104	0.36	0.81	smoker	forklift operator
70.	2018	0.13	0.08	0.45	0.41	non-smoker	anode replacement
71.	2018	0.92	0.31	0.22	0.90	smoker	cell operator
72.	2018	1.68	0.12	0.64	0.91	smoker	anode replacement
73.	2018	0.73	0.38	0.89	3.12	non-smoker	cell start
74.	2018	0.06	0.03	0.18	0.28	smoker	preparation of smelting in the foundry, transport of dross
75.	2018	0.19	0.02	0.47	1.80	smoker	pumping and transport of liquid metal
76.	2018	0.53	0.20	2.09	2.93	smoker	pumping and transport of liquid metal

References

1. International Aluminium Institute. Available online: http://www.world-aluminium.org/statistics/ (accessed on 1 September 2020).
2. Berdowski, J.; van der Most, P.; Mulder, W.; Bloos, J.P.; Payna, J.M.; Rentz, O.; Oertel, D.; Woodfield, M.; Pulles, T.; Appelman, W. Aluminium production. In *2016 EMEP/EEA Emission Inventory Guidebook*; European Environment Agency: Copenhagen, Denmark, 2016.
3. Kvande, H. The aluminium smelting process. *J. Occup. Environ. Med.* **2014**, *56* (Suppl. S5), S2–S4. [CrossRef] [PubMed]
4. European Chemicals Agency. Available online: https://echa.europa.eu/documents/10162/c53c81ed-9e4e-4cb7-9930-f22ca736708c (accessed on 9 April 2020).
5. Ranjan, R.; Ranjan, A. *Fluoride Toxicity in Animals*, 1st ed.; Springer International Publishing: New York, NY, USA, 2015; pp. 11–20.
6. Susheela, A.K.; Mondai, N.K.; Singh, A. Exposure to fluoride in smelter workers in a primary aluminium industry in India. *Int. J. Occup. Environ. Med.* **2013**, *4*, 61–72. [PubMed]
7. Villa, A.; Anabalon, M.; Zohouri, V.; Maguire, A.; Franco, A.M.; Rugg-Gunn, A. Relationships between fluoride intake, urinary fluoride excretion and fluoride retention in children and adults: An analysis of available data. *Caries Res.* **2010**, *44*, 60–68. [CrossRef] [PubMed]
8. Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Fluorides, Hydrogen Fluoride, and Fluorine*; U.S. Department of Health and Human Services, Public Health Service: Atlanta, GA, USA, 2003.
9. Seixas, N.S.; Cohen, M.; Zevenbergen, B.; Cotey, M.; Carter, S.; Kaufam, J. Urinary fluoride as an exposure index in aluminium smelting. *J. Sci. Occup. Environ. Health Saf.* **2010**, *61*, 89–94.
10. Aylward, L.L.; Hays, S.M.; Vezina, A.; Deveau, M.; St-Amand, A.; Nong, A. Biomonitoring Equivalents for interpretation of urinary fluoride. *Regul. Toxicol. Pharmacol.* **2015**, *72*, 158–167. [CrossRef] [PubMed]
11. Idowu, O.S.; Azevedo, L.B.; Valentine, R.A.; Swan, J.; Vasantavada, P.V.; Maguire, A. The use of urinary fluoride excretion to facilitate monitoring fluoride intake: A systematic scoping review. *PLoS ONE* **2019**, *14*, e0222260. [CrossRef]
12. Schwarz, M. Monitoring of fluorine in foodstuffs within the district of Žiar nad Hronom in 1986–1997. *Food Res. Inst. Bratisl.* **1998**, *37*, 53–64.
13. Koç, E.; Karademir, B.; Soomro, N.; Uzun, F. The Effects, both separate and interactive, of smoking and tea consumption on urinary fluoride levels. *Fluoride* **2018**, *51*, 84–96.
14. Kocyigit, A.; Erel, O.; Gur, S. Effects of tobacco smoking on plasma selenium, zinc, copper and iron concentrations and related antioxidative enzyme activities. *Clin. Biochem.* **2001**, *34*, 629–633. [CrossRef]
15. Özden, T.A.; Gökçay, G.; Ertem, H.V.; Süoğlu, Ö.D.; Kılıç, A.; Sökücü, S. Elevated hair levels of cadmium and lead in school children exposed to smoking and in highways near schools. *Clin. Biochem.* **2007**, *40*, 52–56. [CrossRef]
16. Slov-Lex Legislative and Information Portal. Ordinance No. 355/2006 on Protection of Employees against Risks due to Exposure to Chemical Factors at Work. Available online: https://www.slov-lex.sk/pravne-predpisy/SK/ZZ/2006/355/20201001 (accessed on 26 December 2020).
17. National Institute for Occupational Safety and Health. Fluorides, aerosol and gas by ISE. In *1994 Method: 7902, Issue 2*, 4th ed.; NIOSH: Washington, DC, USA, 1994.
18. National Institute for Occupational Safety and Health. Fluoride in Urine. In *1994 Method: 8308, Issue 2*; NIOSH: Washington, DC, USA, 1994.

19. Barr, D.B.; Wilder, L.C.; Caudill, S.P.; Gonzalez, A.J.; Needham, L.L.; Pirkle, J.L. Urinary creatinine concentrations in the U.S. population: Implications for urinary biologic monitoring measurements. *Environ. Health Perspect.* **2005**, *113*, 192–200. [CrossRef] [PubMed]
20. National Institute for Occupational Safety and Health. *Occupational Exposure to Inorganic Fluorides*; NIOSH: Washington, DC, USA, 1976; Publication No. 76103.
21. National Institute for Occupational Safety and Health. *Occupational Exposure to Hydrogen Fluoride*; NIOSH: Washington, DC, USA, 1976; Publication No. 76143.
22. Ehrnebo, M.; Ekstrand, J. Occupational fluoride exposure and plasma fluoride levels in man. *Int. Arch. Occup. Environ. Health* **1986**, *58*, 179–190. [CrossRef] [PubMed]
23. Aarhaug, T.A.; Ratvik, A.P. Aluminium Primary Production Off-Gas Composition and Emissions: An Overview. *JOM* **2019**, *71*, 2966–2977. [CrossRef]
24. Occupational Safety and Health Administration (OSHA). Available online: https://www.osha.gov/annotated-pels/table-z-1 (accessed on 9 December 2020).
25. European Commission Website. Available online: https://ec.europa.eu/health/scientific_committees/opinions_layman/fluoridation/en/l-3/4.htm (accessed on 10 December 2020).
26. Slovak Hydrometeorological Institute. Available online: http://www.shmu.sk/File/oko/rocenky/SHMU_Air_Pollution_in_the_SR_2019_v1.pdf (accessed on 10 December 2020).
27. Pierre, F.; Baruthio, F.; Diebold, F.; Biette, P. Effect of different exposure compounds on urinary kinetics of aluminium and fluoride in industrially exposed workers. *Occup. Environ. Med.* **1995**, *52*, 396–403. [CrossRef]
28. Kono, K.; Yoshida, Y.; Yamagata, H.; Watanabe, M.; Shibuya, Y.; Doi, K. Urinary fluoride monitoring of industrial hydrofluoric acid exposure. *Environ. Res.* **1987**, *42*, 415–520. [CrossRef]
29. Waugh, D.T.; Godfrey, M.; Limeback, H.; Potter, W. Black Tea Source, Production, and Consumption: Assessment of Health Risks of Fluoride Intake in New Zealand. *J. Environ. Public Health* **2017**, *2017*, 1–27. [CrossRef]
30. Whitford, G.M. The Metabolism and Toxicity of Fluoride. *Monogr. Oral Sci.* **1996**, *16*, 1–153. [CrossRef]
31. World Health Organization. *Basic Methods for Assessing Renal Fluoride Excretion in Community Prevention Programmes for Oral Health*; World Health Organization: Geneva, Switzerland, 2014; Available online: https://apps.who.int/iris/handle/10665/112662 (accessed on 6 December 2020).
32. Rees, D.; Rama, D.B.; Yousefi, V. Fluoride in workplace air and in urine of workers concentrating fluorspar. *Am. J. Ind. Med.* **1990**, *17*, 311–320. [CrossRef]
33. Zohouri, F.V.; Swinbank, C.M.; Maguire, A.; Moynihan, P.J. Is the fluoride/creatinine ratio of a spot urine sample indicative of 24-h urinary fluoride? *Community Dent. Oral. Epidemiol.* **2006**, *34*, 130–138. [CrossRef]
34. Centers for Disease Control and Prevention (CDC). Available online: https://www.cdc.gov/niosh/docs/2003-154/pdfs/8308.pdf (accessed on 8 December 2020).
35. Lauwerys, R.R.; Hoet, P. *Industrial Chemical Exposure Guidelines for Biological Monitoring*, 3rd ed.; CRC Press, Inc.: Boca Raton, FL, USA, 2001; pp. 97–102.
36. National Research Council. *Fluoride in Drinking Water: A Scientific Review of EPA's Standards*, 1st ed.; The National Academies Press: Washington, DC, USA, 2006. [CrossRef]
37. Laisalmi, M.; Soikkeli, A.; Kokki, H.; Markkanen, H.; Yli-Hankala, A.; Rosenberg, P.; Lindgren, L. Fluoride metabolism in smokers and non-smokers following enflurane anaesthesia. *Br. J. Anaesth.* **2003**, *91*, 800–804. [CrossRef]
38. Radon, K.; Nowak, D.; Szadkowski, D. Lack of Combined Effects of Exposure and Smoking on Respiratory Health in Aluminium Potroom Workers. *Occup. Environ. Med.* **1999**, *56*, 468–472. [CrossRef] [PubMed]
39. Tu, J.; Liu, K.; Ran, L.; Pan, X. Fluoride burden of aluminium plant workers. *Iran. J. Public Health* **2015**, *44*, 583–585. [PubMed]