Occupational Safety and Hygiene III – Arezes et al. (Eds)
© 2015 Taylor & Francis Group, London, ISBN 978-1-138-02765-7

Genotoxic assessment in different exposure groups working with antineoplastic agents

C. Ladeira & S. Viegas
Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Portugal
CMDT, Universidade Nova de Lisboa, Portugal

M. Pádua & E. Carolino
Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Portugal

M.C. Gomes
Faculty of Sciences, University of Lisbon, Portugal

M. Brito
Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Portugal

ABSTRACT: Antineoplastic drugs are widely used in treatment of cancer, and several studies suggest acute and long-term effects associated to antineoplastic drug exposures, namely associating workplace exposure with health effects. Cytokinesis Blocked Micronucleus (CBMN) assay is one promising short-term genotoxicity assays for human risk assessment and their combination is recommended to monitor populations chronically exposed to genotoxic agents. The aim of this investigation is the genotoxicity assessment in different professionals that handle cytostatics drugs. This research is case-control blinded study constituted by 46 non-exposed subjects and 44 workers that handle antineoplastic drugs, such as pharmacists, pharmacy technicians, and nurses. It was found statistically significant increases in the genotoxicity biomarkers in exposed comparising with controls ($p < 0.05$). The findings address the need for regular biomonitoring of personnel occupationally exposed to these drugs, confirming to an enhanced health risk assessment.

1 INTRODUCTION

Antineoplastic or cytostatics drugs are a heterogeneous group of chemicals widely used in the treatment of cancer and in some non-neoplasic diseases, having in common an ability to inhibit tumour growth by disrupting cell division and killing actively growing cells. These drugs have nevertheless been proved to be also mutagens, carcinogens and teratogens (Gulten, 2011).

In general, chemicals that interact directly with DNA by binding covalently or by intercalating, or indirectly by interfering with DNA synthesis, were among the first chemotherapeutics developed. Compounds that inhibit mitotic spindle formation and those that affect endocrine function are also used in cancer chemotherapy. Also, these drugs can induce reactive oxygen species that can lead to DNA damage and, consequently, mutations.

Accordingly, several antineoplastic drugs have been classified by the International Agency for Research on Cancer (IARC), on the basis of epidemiological reports, animal carcinogenicity data, and the outcomes of *in vitro* genotoxicity studies, as belonging to the group of human carcinogens (Group 1), probable human carcinogens (Group 2 A), or possible human carcinogens (Group 2B). In addition, investigational agents have also to be considered as potentially hazardous until their safety can be established. According to European Guidelines (Corrigendum to Directive 2004/37/EG), any use of carcinogenic, mutagenic or teratogenic substances, including the application in health care settings, are assigned to the highest risk level (Gulten et al., 2011).

There are several studies that suggest both acute and long-term health effects associated to antineoplastic drug exposures, and various studies have associated workplace exposure with health effects such as skin rashes, hair loss, irritation, hypersensitivity, and headaches after reported skin contact. Negative reproductive health outcomes are also associated with antineoplastic exposure. Spontaneous abortions have been reported approximately twice

more often among exposed pregnancies than unexposed ones; the same goes for congenital malformations, infertility, and possibly leukemia, as well as other cancers (Kopjar et al., 2009).

Health care workers who prepare or administer hazardous drugs or who work in areas where these drugs are used may be exposed to these agents in the air, on work surfaces, contaminated clothing, medical equipment, patient excreta, and other surfaces (Kopjar et al., 2009). Exposures may occur through inhalation resulting from aerosolization of powder or liquid during reconstitution and spillage taking place while preparing or administering to patients, through skin contact, skin absorption, ingestion, or injection. Inhalation and skin contact/absorption are the most likely routes of exposure, but unintentional ingestion from hand to mouth contact and unintentional injection through a needle stick or sharps injury are also possible (Kopjar et al., 2009; El-Ebiary et al., 2011). Hand contact with contaminated equipment used in preparing and administering these drugs, or contaminated food or cigarettes, all lead to oral ingestion. Furthermore, patients may excrete these drugs and their metabolic by-products in body wastes, exposing personnel who handle such items (Kopjar et al., 2009; El-Ebiary et al., 2011).

Contamination of the work surfaces and also permeation of gloves to some antineoplastic drugs were reported already in several studies (Kopjar et al., 2009; Gulen, 2011). Moreover, vaporization of spilled antineoplastic drugs may represent an additional route of exposure to healthcare workers through inhalation. However, contact with contaminated surfaces seems to have the most important role in exposure due to dermal absorption. Therefore, the monitoring of surfaces contamination is a common way to assess occupational exposure, being the wipe sampling the most common method used (Hedmer, 2004) allowing for the demonstration of widespread workplace contamination, even when strict protocols and standard operating procedures have been applied.

The monitoring of genotoxic risks should be done combining environmental and biological monitoring with procedures of biological effect monitoring (primary DNA damage and chromosome damage). In this integrated chemical/biotoxicological approach, the use of genotoxicity biomarkers measuring changes in cellular or molecular endpoints (e.g., DNA and/or chromosome damage) will allow us to combine environmental and biological monitoring with biological effect monitoring. Cytokinesis-Blocked Micronucleus (CBMN) assay is recognized technique that can be a predictor of cancer risks in humans, and because of its ability to detect both clastogenic (e.g., chromosome breakage) and aneugenic (e.g., spindle disruption) effects, it could have a role in occupational health surveillance programs for workers exposed to antineoplastic drugs to monitor long-term exposure effects (a so-called biomarker of early/preclinical biological effects). Basically, it can be measured by CBMN assay Micronuclei (MN), biomarker of chromosome loss and breakage; Nucleoplasmic Bridges (NPB), biomarker of chromosome rearrangement, and Nuclear Buds (NBUD), biomarker of gene amplification.

The aim of this study was to assess genotoxic effects and compare it by professional exposed group.

2 METHODS

2.1 *Samples*

As for exposure to cytostatics, the sample of cases comprised 44 workers which have been exposed in two pharmacy laboratories and three nursing hospitals. The control group was formed by 46 subjects from academia who have not been exposed to cytostatics. To all individuals (controls and workers) were asked if they take any prescribed or not prescribed drug or health treatment that can influence the health effects measured. Ethical approval was obtained from the Institutional Ethical Board and Service Director of the hospitals, and all subjects gave informed consent to participate.

2.2 *Cytostatics exposure assessment*

Surfaces contamination was investigated in the two hospitals by wipe sampling in areas where antineoplastic drugs and were prepared administered as recommended by Hedmer et al. (2004). The studied cytostatics—cyclophosphamide, 5-fluorouacil and paclitaxel, were considered suitable indicators for occupational exposure to antineoplastic drugs because are frequently used in preparations and in high amounts in both hospitals considered (Castiglia et al., 2008). Sensitive analytical methods are already established for these drugs. In both hospitals, sampling was developed in two different days. Regarding antineoplastic drug administration, the days were indicated by workers and services as normal working days.

2.3 *Genotoxicity assessment*

Heparinized blood samples were obtained by venipuncture from all subjects and freshly collected peripheral blood was used for the CBMN assay. All samples were coded and analyzed under blind conditions. The criteria for scoring the nuclear abnormalities in lymphocytes were the ones

described by Fenech et al. (1999). All samples were coded and analyzed under blind conditions.

3 RESULTS

Two samples were formed—the group of those occupationally exposed to cytostatics and the non-exposed group (controls). Population characteristics such as gender distribution, age, years of exposure, tobacco and alcohol consumption for the control and exposed groups are shown in Table 1.

The two studied hospitals presented cytostatics contamination with one of the three studied drugs or with more than one drug. The data of cytostatics exposure assessment regarding these units were previously published in Viegas et al., 2014 and Ladeira et al. 2014.

Table 1. Characteristics of the samples.

	Controls	Exposed
Number of subjects	46	44
Gender		
Females	34 (73.91%)	37 (84.09%)
Males	12 (26.09%)	7 (15.91%)
Age (mean ± standard deviation, in years)	38.93 ± 9.70	33.60 ± 8.31
Range	20–61	24–58
Years of exposure (mean ± standard error of mean, in years)	n.a.	6.01
Range	n.a.	0.17–30
Tobacco consumption		
Non-smokers	12 (73.91%)	40 (90.91%)
Smokers	34 (26.09%)	4 (9.09%)
Alcohol consumption		
Non-drinkers	33 (71.74%)	32 (73.73%)
Drinkers	13 (28.26%)	12 (27.27%)

Comparing the control group with the three occupations studied it was found statistical significant differences for some of the genotoxicity biomarkers. In what concern to pharmacists there were statistical significant differences in MN in binucleated lymphocytes, NPB, and NBUD and the control group. Pharmacy technicians presented statistical significant results for MN in bi and mononucleated lymphocytes, and NPB. The nurses group present statistical significant differences in MN in binucleated cells and NPB. No association was found between years of exposure and presence of any of the biomarkers measured by CBMN assay. Regarding demographic and lifestyle habits, MN tended to increase with age whereas gender, alcohol and tobacco consumption did not shown any effect in the genotoxicity frequencies.

4 DISCUSSION AND CONCLUSIONS

Considering surfaces contamination results, positive samples were found for all surrogate markers in both hospitals. These results implicate concern because health effects associated to exposure to carcinogenic, mutagenic, and teratogenic substances usually do not depend on a minimum dose but instead on a prolonged exposure. Therefore, it can be concluded that there is no safety threshold dose concerning exposure to these drugs being appropriate to apply the ALARA principle: keep exposure/contamination levels "As Low As Reasonably Achievable". Widespread contamination was also observed in other studies, despite the implementation of safety procedures for handling antineoplastic drugs (Castiglia et al., 2008).

These results can be explained once again by the fact that there are more strict safety and hygiene rules in preparation units when compared with administration units as demonstrated by the high number of organizations that research on this field

Table 2. Descriptive statistics of MN, NPB and NBUD in the studied population (mean ± mean standard error, range), p-value of the Mann-Whitney test concerning the association between exposure and genotoxicity biomarkers.

Occupation	n	MN BN Mean ± S.E. (range)	NPB Mean ± S.E. (range)	NBUD Mean ± S.E. (range)	MN MONO Mean ± S.E. (range)	MN MULTI Mean ± S.E. (range)
Controls	46	5.09 ± 0.89 (0–34)	0.11 ± 0.05 (0–1)	1.37 ± 0.32 (0–13)	0.41 ± 0.11 (0–3)	1.46 ± 0.22 (0–6)
Pharmacist	11	7.82 ± 1.30 (1–16)	1.09 ± 0.39 (0–3)	2.82 ± 0.58 (0–6)	0.36 ± 0.20 (0–2)	4.82 ± 1.90 (0–21)
P-value		0.029*	0.001*	0.009*	0.804	0.102
Pharmacy technician	6	10.83 ± 2.10 (4–19)	0.50 ± 0.22 (0–1)	2.17 ± 0.70 (0–5)	1.17 ± 0.31 (0–2)	4.50 ± 1.98 (0–12)
P-value		0.010*	0.013*	0.124	0.009*	0.111
Nurse	27	10.11 ± 2.05 (1–58)	0.48 ± 0.15 (0–3)	2.41 ± 0.57 (0–11)	1.78 ± 0.51 (0–9)	3.85 ± 1.02 (0–28)
P-value		0.001*	0.014*	0.073	0.053	0.218

developing constantly new rules and safety measures (NIOSH 2004; ISOPP, 2007, among others). In this case, the inappropriate surfaces cleaning together with the incorrect working procedures probably are contributing for the contamination found.

In what concern to genotoxicity assessment, the results showed statistical significant higher means for some endpoints studied between the three professions groups and controls ($p < 0.05$). These results, namely in what concern to micronuclei frequency, are corroborated by many others studies (Kopjar et al., 2009; El-Ebiary et al., 2011) that found also significant increase of micronuclei frequency in workers handling antineoplastic drugs.

The present study confirmed that there is surface contamination in the workplaces considered and the cytogenetic endpoint studied (CBMN) showed signs of an effect related with exposure. Since the genotoxicity may be due to combined effects of all or some of the antineoplastic drugs, it is not possible to attribute damage to any particular agent. Results of this study as well as previous investigations performed on subjects occupationally exposed to antineoplastic drugs using different genotoxicity endpoints suggest that mixtures of antineoplastic drugs in long-term occupational exposure may act as clastogens on the DNA molecule of somatic cells.

Since no occupational exposure limits have been established for airborne concentrations of antineoplastic drugs and for their concentration in the urine, there is no exposure level can be considered safe, and thus zero contamination should be the target. Exposure to these compounds should be avoided, and safety guidelines and protective measures like wearing masks, gloves, gowns, caps, protective eyewear and the preparation of drugs in biological safety cabinets are normally available in the workplaces in order to prevent exposure (Sessink et al., 1992; Gulen, 2011).

REFERENCES

Castiglia, L., Miraglia, N., Pieri, M., Simonelli, A., Basilicata, P., Genovese, G., Guadagni, R., Acampora, A., Sannolo, N. & Scafarto, M.V. (2008). Evaluation of occupational exposure to antiblastic drugs in an Italian hospital oncological department. *Journal of occupational health*. 50 (1). pp. 48–56.

El-Ebiary, A.A., Abuelfadl, A.A. & Sarhan, N.I. (2013). Evaluation of genotoxicity induced by exposure to antineoplastic drugs in lymphocytes of oncology nurses and pharmacists. *Journal of Applied Toxicology*. 33 (3). p.pp. 196–201.

Fenech, M., Holland, N., Chang, W.P., Zeiger, E. & Bonassi, S. (1999). The Human MicroNucleus Project—An international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. *Mutation research*. 428 (1–2). pp. 271–283.

Gulten, T., Evke, E., Ercan, I., Evrensel, T., Kurt, E. & Manavoglu, O. (2011). Lack of genotoxicity in medical oncology nurses handling antineoplastic drugs: effect of work environment and protective equipment. *Work (Reading, Mass.)*. 39 (4). pp. 485–489.

Hedmer, M., Jönsson, B.A.G. & Nygren, O. (2004). Development and validation of methods for environmental monitoring of cyclophosphamide in workplaces. *Journal of Environmental Monitoring*. 6 (12). pp. 979–984.

Kopjar, N., Garaj-Vrhovac, V., Kašuba, V., Rozgaj, R., Ramić, S., Pavlica, V. & Želježić, D. (2009). Assessment of genotoxic risks in Croatian health care workers occupationally exposed to cytotoxic drugs: A multibiomarker approach. *International Journal of Hygiene and Environmental Health*. 212 (4). pp. 414–431.

Ladeira, C., Viegas, S., Pádua, M., Gomes, M., Carolino, E., Gomes, M.C. & Brito, M. (2014). Assessment of Genotoxic Effects in Nurses Handling Cytostatic Drugs. *Journal of Toxicology and Environmental Health, Part A*. 77 (14–16). pp. 879–887.

Sessink, P.J., Boer, K.A., Scheefhals, A.P., Anzion, R.B. & Bos, R.P. (1992). Occupational exposure to antineoplastic agents at several departments in a hospital. Environmental contamination and excretion of cyclophosphamide and ifosfamide in urine of exposed workers. *International archives of occupational and environmental health*. 64 (2). pp. 105–112.

Viegas, S., Pádua, M., Veiga, A.C., Carolino, E. & Gomes, M. (2014). Antineoplastic drugs contamination of workplace surfaces in two Portuguese hospitals. *Environmental Monitoring and Assessment*. [Online]. Available from: http://link.springer.com/10.1007/s10661-014-3969-1.

Occupational Safety and Hygiene III – Arezes et al. (Eds)
© 2015 Taylor & Francis Group, London, ISBN 978-1-138-02765-7

Assessment of children exposure doses to ultrafine particles in primary schools

J. Cavaleiro-Rufo, J. Madureira & E. de Oliveira Fernandes
Institute of Mechanical Engineering and Industrial Management, Porto, Portugal

M. Pinto & A. Moreira
Faculty of Medicine of the University of Porto, Porto, Portugal

K. Slezakova & M.C. Pereira
Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering of the University of Porto, Porto, Portugal

C. Pereira & J.P. Teixeira
National Institute of Health, Porto, Portugal

ABSTRACT: Children attending primary schools may be largely exposed to the Ultrafine Particles (UFPs) present in the classroom's indoor air, which may lead to severe health consequences resultant from their increased susceptibility. Thus, this study aimed to estimate the UFP exposure dose rates in Portuguese children attending public primary schools. Ultrafine particles were sampled in 10 public primary schools located in Porto. Exposure dose rates were estimated for 488 children (aged 8 to 10 years) of the sampled schools. The estimated mean of exposure dose rates in children were $4.06 \times 10^8 \pm 0.11 \times 10^8$ part/kg.day. Specific indoor activities as well as outdoor environment conditions appeared to be associated with increased indoor UFP number concentrations and, consequently, with higher exposure doses in children attending those schools. Overall, children showed at least two times higher UFP exposure dose rates when compared to occupationally exposed adults (i.e. teachers and school staff).

1 INTRODUCTION

Currently, several guidelines and strategies have been developed in order to reduce the health risk caused by indoor exposure to particulate matter (WHO, 2005). For instance, the EnVIE project showed that source control approaches and adequate ventilation are the key elements to reduce health problems related with inadequate indoor air quality, including fine and coarse particulate matter pollution (Oliveira Fernandes, 2008). However, there are no regulations regarding the concentrations of Ultrafine Particles (UFPs) which are particles smaller <0.1 μm and s strong source of oxidative stress and lung inflammation, possibly causing the onset or exacerbation of asthma and other respiratory diseases. The strong toxicity of UFPs is often associated to their proficiency for penetrating cell membranes (Peters et al., 1997, Penttinen et al., 2001) and consequent carcinogenic activity (Stanek et al., 2011).

In Portugal, children attendance in primary school is compulsory and, in general, they spend at least 7 hours per day in these institutions, from Monday to Friday, which may be reflected as a large period of exposure to indoor UFPs. Moreover, children tend to be more susceptible to UFPs toxicity particularly due to their immature respiratory systems, reduced constitution and minor lung function (Schwartz, 2004). To evaluate the health risk resulting from UFP pollution, several studies have been assessing the dose rates of exposure to these particles in children (Buonanno et al., 2012, 2013, Mazaheri et al., 2014). Fonseca et al. (2014) reported UFP inhalation exposure doses for children estimated at 3 Portuguese preschools; the results showed that 3 to 5 years old children presented 4 to 6 times higher dose rates than adults with similar daily schedules. Since children in primary schools spend more time than those in preschools, it is possible that the exposure dose rates to indoor UFPs are also higher in these indoor environments, even if similar particle number concentrations are considered.

Therefore, the aim of this study was to estimate the UFP inhalation dose rates in Portuguese

children (8–10 years old) attending public primary schools. Additional objectives of this work were: a) to estimate the exposure doses of UFPs in adults with similar schedule conditions; and b) to compare the different exposure doses between the populations of children and adults.

2 MATERIALS AND METHODS

2.1 *Sampling*

Ultrafine particles were sampled in 10 public primary schools (35 classrooms) located in the urban area of Porto (S1 to S10) during the heating season (between January and April 2014). More detailed information regarding the sampling sites is shown in Table 1.

The measurements were performed during a regular school day and under representative activities, conditions of occupancy and use of the classrooms (from 9:00 to 16:00). In general, the recess periods occurred from 10:30 to 11:00 and from 12:30 to 14:00. These were non-occupation periods and were not consider for the estimation of the exposure dose rates.

Two portable condensation particle counters (P-Track model 8525, TSI Inc., MN, USA) were used for the assessment of UFP number concentrations. The instruments were installed inside each classroom and were set to continuously measure during at least one school day. Logging intervals were set to 1 minute between each sample according to previously published studies (Norback et al., 2011, Zhang and Zhu, 2012, Fonseca et al., 2014). Further detailed characterization of the equipment has been previously reported (Matson et al., 2004).

The instruments were mounted on a flat surface at the height of the children's breathing zone (1.2 to 1.5 m) as far as possible from windows or doors as well as from major indoor sources of UFPs. A researcher supervised the sampling process and recorded relevant information.

2.2 *Exposure dose rate calculation*

In this study, 713 children attended the sampled classrooms. The children' legal guardians received an envelope with information regarding the project as well as a written consent form, in accordance with the Helsinki declaration. The UFP exposure dose rates were calculated for all 488 (aged 8 to 10 years old) that were authorized to participate in the study (66.8%). Children's UFP exposure dose rates was calculated using the age and body weight-specific formula presented as Equation 1, which has been validated in previously published studies (Ginsberg et al., 2005, Kalaiarasan et al., 2009, Castro et al., 2011, Fonseca et al., 2014).

$$D = \left(\frac{BR_{WA}}{BW}\right) \times C_{WA} \times OF \times N \quad (1)$$

In this equation, D represents the age-specific dose rate (part./kg/day); BR_{WA} is the age-specific

Table 1. Main characteristics of the sampled schools.

School	Building location	Cooking	Number of sampled classrooms	Classroom ventilation
S1	Low traffic; mixed residential and industrial area.	No	2	Natural ventilation (small grids; inoperable windows)
S2	Low traffic; residential area.	No	4	Natural ventilation (not facing the main street)
S3	High traffic; densely packed residential area.	No	4	Natural ventilation (windows do not face the main street)
S4	Low traffic; residential area; hospital in the proximity.	No	4	Natural ventilation (windows do not face the main street)
S5	Medium traffic and metro; densely packed residential area.	Yes	4	Natural ventilation (not facing the main street)
S6	High traffic; densely packed residential area.	No	4	Natural ventilation (facing the main street)
S7	Low traffic; densely packed residential area.	No	4	Natural ventilation (windows do not face the main street)
S8	Medium traffic; densely packed residential area.	No	2	Natural ventilation (windows do not face the main street)
S9	Medium traffic; densely packed residential area.	No	3	Natural ventilation
S10	High traffic and metro; densely packed residential area.	No	4	Natural ventilation

weighted average breathing rate (L/min); BW is the body weight of the children (kg); C_{WA} is the weighted average particle number concentrations (part./L); OF is the occupancy factor; N is the total time per day spent in the location of exposure (min/day).

The BR_{WA} is characterized by the intensity of the activity practiced at the time of exposure. Seeing as primary schoolchildren are normally seated during the time they spend in classroom (writing, studying, drawing, etc.), the "sedentary/passive" activity level was selected. The age-specific inhalation factors were retrieved from the US EPA exposure factors handbook (U.S. Environmental Protection Agency, 2011). Thus, BR_{WA} was considered as 4.8 L/min for 8 to 10 years old children. The BW of children was determined by a certified body composition analyzer (Tanita® TBF-300 A, capacity 200 kg, accuracy 100 g) operated by a trained nurse. Children were weighted barefooted and 1 kg was deducted from the measured weight to account the clothing. The platform and handle electrodes of the scale were cleansed with 96% alcohol after each measurement. C_{WA} was estimated using the UFP average number concentrations weighted by the real time that children spent inside the classroom and the OF was always considered as 1, since children kept their schedules and their respective locations tightly. Although children are compelled to stay at least 7 hours in primary schools, the total time per day spent inside the classroom represents only 5 hours of that time period since they have 2 hours of recesses each day. Therefore, N was considered as 300 min/day (5 hours).

For comparison purposes, UFP exposure dose rates were also estimated for adults in similar conditions (aged 21 to 60 years old). When concerning "sedentary/passive" activities, the BR_{WA} in adults was considered 4.2 L/min for ages between 21 and 30, 4.3 L/min between 31 and 40, 4.8 L/min between 41 and 50, and 5.0 L/min between 51 and 60 (U.S. Environmental Protection Agency, 2011). The average BW considered for all groups of adults was 70.8 kg according to the European region average body mass in 2005 (Walpole et al., 2012).

Statistical analysis was performed using SPSS Statistics v20 (IBM). Statistical significance was considered when $p < 0.05$.

3 RESULTS AND DISCUSSION

The statistical analysis showed no significant differences regarding BW between both genders ($p = 0.748$). Moreover, there were also no significant BW differences between the 10 schools ($p = 0.156$). These results suggest that, despite the differences in gender and location of the school, there are no major dissimilarities in the average BW of 8 to 10 years old primary schoolchildren.

The total mean (average and standard error of the mean) of UFP inhalation dose rates in children (8 to 10 years old) of 10 primary schools was $4.06 \times 10^8 \pm 0.11 \times 10^8$ part./kg·day. The information regarding the average inhalation dose rates in each school is summarized in Table 2. The results showed that exposure doses in children attending S5 were 1.2 to 4.8 times higher than those of other schools. These results may be associated with the increased production of UFPs resulting from the performed cooking activities; S5 was the only school in this study where meals for the children were directly cooked in the respective school building. These findings support previously published studies showing that cooking practices are significantly associated with increased UFP

Table 2. Average dose rates (D, part/kg.day) per school and age group.

School	Mean UFP number concentrations (part./cm³)	Average UFP inhalation dose rates (part/kg·day)				
		Children (8 to 10)	Adults (21 to 30)	Adults (31 to 40)	Adults (41 to 50)	Adults (51 to 60)
S 1	3.37×10^3	1.48×10^8	6.00×10^7	6.15×10^7	6.86×10^7	7.15×10^7
S 2	6.58×10^3	2.96×10^8	1.17×10^8	1.20×10^8	1.34×10^8	1.39×10^8
S 3	5.68×10^3	2.65×10^8	1.01×10^8	1.03×10^8	1.16×10^8	1.20×10^8
S 4	7.35×10^3	3.17×10^8	1.31×10^8	1.34×10^8	1.50×10^8	1.56×10^8
S 5	1.49×10^4	7.06×10^8	2.65×10^8	2.71×10^8	3.03×10^8	3.16×10^8
S 6	1.25×10^4	5.85×10^8	2.23×10^8	2.28×10^8	2.55×10^8	2.66×10^8
S 7	8.78×10^3	3.71×10^8	1.56×10^8	1.60×10^8	1.79×10^8	1.86×10^8
S 8	7.61×10^3	3.43×10^8	1.35×10^8	1.39×10^8	1.55×10^8	1.61×10^8
S 9	8.36×10^3	3.76×10^8	1.49×10^8	1.52×10^8	1.70×10^8	1.77×10^8
S 10	9.21×10^3	4.26×10^8	1.64×10^8	1.68×10^8	1.87×10^8	1.95×10^8

S—School; UFP—Ultrafine particles.

production (Zhang and Zhu, 2012). Schools S6 and S10 also presented dose rates above the average value, probably due to the closer proximity to roads with intense traffic, which are considered major sources of ambient UFPs that may penetrate to the indoor environment of the schools (Kulmala et al., 2004). Finally, S1 exhibited 1.8 to 4.8 lower UFP inhalation dose rates than the estimated mean. The lower traffic intensity in the school area and the inoperable windows (ventilation only promoted by small grids) may hinder the penetrance of UFPs from the outdoors, which consequently could lead to lower dose rates in S1.

To further comprehend the extent of UFP exposure in the analyzed schools, the dose rates of children were compared with those of adults. The results showed that the UFP dose rates increased with the increasing age of adults, being minimal in individuals aged from 21 to 30 years old and constantly rising as the age increases due to the higher inhalation dose rates, which is probably associated to the natural lung function loss resulting from the aging process (Harik-Khan et al., 1998). However, due to the children vulnerability and small body weight, they still showed 2.0 to 2.2 higher UFP exposure doses than the older adults (51–60). These results demonstrate the high susceptibility of children to UFPs and indicate that primary schools have an important role in the child's overall particle exposure.

Information regarding primary school children exposure doses to UFPs is scarce. In addition, the different study approaches and the dissimilar characteristics of the sampled indoor microenvironments hamper further comparisons. Therefore, the dose rates estimated within this work were not compared with other studies.

4 CONCLUSIONS

This is the first study estimating the UFP exposure dose rates for 8 to 10 years old children attending Portuguese primary schools. The results suggest that schools located near busy roads as well as schools with meals directly cooked in the building have higher indoor number concentrations of UFPs. Nevertheless, to further study the children total exposure in schools, the UFP exposure dose rates should be assessed in other areas, such as canteens and/or gymnasiums.

Children susceptibility to UFP exposure was supported by this study and, when compared to adult individuals in similar conditions, they had at least two times higher dose rates, even when compared to older individuals. Therefore, special attention should be given to the major sources of ultrafine particles in primary schools in order to minimize the exposure dose rates in children. Further investigations regarding building characteristics and sources of UFPs would be important to provide information to protect public health.

ACKNOWLEDGMENTS

This study was performed in the framework of the ARIA project financed by Fundação para a Ciência e Tecnologia (PTDC/DTP-SPA/1522/2012, FCOMP-01-0124-FEDER-028709) and the fellowship SFRH/BPD/65722/2009.

REFERENCES

Buonanno, G., Marini, S., Morawska, L. & Fuoco, F. C. 2012. Individual dose and exposure of Italian children to ultrafine particles. *Sci Total Environ,* 438, 271–7.

Buonanno, G., Marks, G.B. & Morawska, L. 2013. Health effects of daily airborne particle dose in children: direct association between personal dose and respiratory health effects. *Environ Pollut,* 180, 246–50.

Castro, D., Slezakova, K., Delerue-Matos, C., Alvim-Ferraz, M. d. C., Morais, S. & Pereira, M. d. C. 2011. Polycyclic aromatic hydrocarbons in gas and particulate phases of indoor environments influenced by tobacco smoke: Levels, phase distributions, and health risks. *Atmospheric Environment,* 45, 1799–1808.

Fonseca, J., Slezakova, K., Morais, S. & Pereira, M. C. 2014. Assessment of ultrafine particles in Portuguese preschools: levels and exposure doses. *Indoor Air,* n/a-n/a.

Ginsberg, G.L., Foos, B.P. & Firestone, M.P. 2005. Review and analysis of inhalation dosimetry methods for application to children's risk assessment. *J Toxicol Environ Health A,* 68, 573–615.

Harik-Khan, R.I., Wise, R.A. & Fozard, J.L. 1998. Determinants of maximal inspiratory pressure. The Baltimore Longitudinal Study of Aging. *Am J Respir Crit Care Med,* 158, 1459–64.

Kalaiarasan, M., Balasubramanian, R., Cheong, K.W.D. & Tham, K.W. 2009. Traffic-generated airborne particles in naturally ventilated multi-storey residential buildings of Singapore: Vertical distribution and potential health risks. *Building and Environment,* 44, 1493–1500.

Kulmala, M., Vehkamäki, H., Petäjä, T., Dal Maso, M., Lauri, A., Kerminen, V.M., Birmili, W. & McMurry, P.H. 2004. Formation and growth rates of ultrafine atmospheric particles: a review of observations. *Journal of Aerosol Science,* 35, 143–176.

Matson, U., Ekberg, L. E. & Afshari, A. 2004. Measurement of Ultrafine Particles: A Comparison of Two Handheld Condensation Particle Counters. *Aerosol Science and Technology,* 38, 487–495.

Mazaheri, M., Clifford, S., Jayaratne, R., Megat Mokhtar, M.A., Fuoco, F., Buonanno, G. & Morawska, L. 2014. School children's personal exposure to ultrafine particles in the urban environment. *Environ Sci Technol,* 48, 113–20.

Norback, D., Wieslander, G., Zhang, X. & Zhao, Z. 2011. Respiratory symptoms, perceived air quality and physiological signs in elementary school pupils in relation to displacement and mixing ventilation system: an intervention study. *Indoor Air,* 21, 427–37.

Oliveira Fernandes, E., Gustafsson, H., Seppänen, O., Crump, D. & Ventura Silva, G. 2008. WP3 Final Report on Characterization of Spaces and Sources. EnVIE Project. *Brussels: European Commission 6th Framework Programme of Research.*

Penttinen, P., Timonen, K.L., Tiittanen, P., Mirme, A., Ruuskanen, J. & Pekkanen, J. 2001. Number concentration and size of particles in urban air: effects on spirometric lung function in adult asthmatic subjects. *Environ Health Perspect,* 109, 319–23.

Peters, A., Wichmann, H.E., Tuch, T., Heinrich, J. & Heyder, J. 1997. Respiratory effects are associated with the number of ultrafine particles. *Am J Respir Crit Care Med,* 155, 1376–83.

Schwartz, J. 2004. Air pollution and children's health. *Pediatrics,* 113, 1037–43.

Stanek, L.W., Sacks, J.D., Dutton, S.J. & Dubois, J.-J. B. 2011. Attributing health effects to apportioned components and sources of particulate matter: An evaluation of collective results. *Atmospheric Environment,* 45, 5655–5663.

U.S. Environmental Protection Agency 2011. *Exposure Factors Handbook 2011 Edition (Final)*, Washington, DC, U.S. Environmental Protection Agency.

Walpole, S.C., Prieto-Merino, D., Edwards, P., Cleland, J., Stevens, G. & Roberts, I. 2012. The weight of nations: an estimation of adult human biomass. *BMC Public Health,* 12, 1471–2458.

WHO. 2005. WHO Air quality guidelines for particulate matter, ozone, nitrogen dioxide and sulfur dioxide.

Zhang, Q. & Zhu, Y. 2012. Characterizing ultrafine particles and other air pollutants at five schools in South Texas. *Indoor Air,* 22, 33–42.