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ORIGINAL ARTICLE

Hydroxypyrene in urine of football players after playing on artificial sports field with tire crumb infill

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Abstract

Background Artificial sports fields are increasingly being used for sports. Recycled rubber from automotive and truck scrap rubber tires are used as an infill material for football grounds. There are concerns that football players may be at risk due to exposure from released compounds from rubber infill. Compounds from crumb infill may be inhaled and dermal exposure may occur. A study was performed to assess the exposure of football players to polycyclic aromatic hydrocarbons due to sporting on synthetic ground with rubber crumb infill.

Methods In this study, football players were trained and had a match on the artificial turf pitch during 2.5 h. They had an intensive skin contact with rubber infill. All urine of seven nonsmoking football players was collected over a 3-day period, the day before sporting, the day of sporting and the day after sporting. Urine samples were analyzed for 1-hydroxypyrene. Confounding exposure from environmental sources and diet was controlled for.

Results The individual increase of the amount of excretion over time was used as a measure to assess the uptake of PAH. It appeared that the baseline of excreted 1-hydroxypyrene in 4 of 7 volunteers was sufficient stable and that 1 volunteer out of 4 showed after the 2.5-h period of training and match on the playground an increase in hydroxypyrene in urine. However, concomitant dietary uptake of PAH by this volunteer was observed.

Conclusions This study provides evidence that uptake of PAH by football players active on artificial grounds with rubber crumb infill is minimal. If there is any exposure, than the uptake is very limited and within the range of uptake of PAH from environmental sources and/or diet.

Keywords 1-Hydroxypyrene · Rubber crumb infill · Football players · Biological monitoring

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Introduction

Artificial, synthetic grass fields for sporting are increasing being used. Recycled rubber from automotive and truck rubber tires are used as an infill material for football fields. Crumb rubber is a term usually applied to recycled rubber from tires.

Vulcanized rubber tire is well recognized as a material with many highly toxic additives and compounds. Hazardous substances in crumb rubber infill are primarily heavy metals (especially zinc), volatile components (nitrosamines, xylenes), benzothiazoles, secondary amines and polycyclic aromatic hydrocarbons (PAHs). The zinc is due to zinc oxide that is used as a vulcanization aid in the rubber production process. PAHs are from high-aromatic oil that is used as an additive in the production of tires. Nitrosoamines are formed during the vulcanization process. Xylene is a solvent. Benzothiazoles are accelerators in the vulcanization process, and the secondary amines are antioxidants for the rubber.

Concern has been expressed on health effects of sporters active playing on this type of field, due to the exposure of releasing constituents from the rubber crumb. Sporters playing on such synthetic grounds might be exposed to constituents due to inhalation of volatiles and/or particles from the rubber crumb and due to dermal contact with the rubber crumb grains.

Polycyclic aromatic hydrocarbons (PAHs) may be released from the rubber infill into the air and water. The release of PAHs from crumb has been studied via leaching in laboratory experiments. Risk assessments have been made assuming a certain intake by inhalation, dermal contact and ingestion (Birkholz et al. 2003; Zhang et al. 2008). The assessment of the risk of PAH uptake of sporters has been assessed as low (Hofstra 2007), assuming a certain exposure scenario and dermal bioavailability. However, real life exposure testing of sporters active on artificial turf has not yet been performed.

When exposure can occur through multiple pathways, the assessment can best be done using a biological monitoring method. Urinary 1-hydroxypyrene is a useful and widely used quantitative biological indicator of exposure to PAHs, reflecting both absorbed dose and body burden (Jongeneelen et al. 1985; Tolos et al. 1990; Buchet et al. 1992; WHO 1996; Bouchard and Viau 1999; ACGIH 2005; Unwin et al. 2006). Some excellent reviews have been published (Dor et al. 1999; Bouchard and Viau 1999; Hansen et al. 2008). Studies of carbon black workers show that 1-hydroxypyrene is a useful indicator of occupational exposure to the PAH adsorbed onto carbon black (Gardiner et al. 1992; Tsai et al. 2002). In exposed workers in rubber production, an increased level of urinary hydroxypyrene was found (Peters et al. 2008).

The urine test is specific for exposure to pyrene, which is always present in the mixture of PAH. Pyrene itself is not genotoxic, but the PAH mixture contains many other genotoxic PAH. A certain background concentration of 1-hydroxypyrene is found in urine due to the intake of traces of PAH through the diet and due to environmental sources, such as environmental tobacco smoke, diesel engine exhaust and urban outdoor air. Van Rooij et al. (1994) studied the sources of interindividual variation of urinary 1-hydroxypyrene. They concluded that smoking and dietary intake of PAH are the major factors for variation.

There is a growing awareness that the PAH uptake via the skin can be very substantial. It has become clear that dermal exposure of PAH may not only result in local effects in the skin but also result in systemic effects. PAH–DNA adducts were found in lungs after dermal application of tar and bitumen products on skin of mice (Schoket et al. 1988). A series of studies show that the dermal exposures can be traced and quantified using the urinary 1-hydroxypyrene assay (Van Rooij et al. 1992; Van Rooij et al. 1993; Viau et al. 1995; Elovaara et al. 1995).

This paper reports a study of the uptake of PAH by football players due to playing on a synthetic crumb rubber infill playing ground. Urine of the players was tested for 1-hydroxypyrene.

Materials and methods

Design of the biomonitoring survey

Seven male, nonsmoking football players of team A6 of SV Juliana'31 in Malden NL, volunteered to participate in the study. The characteristics of the volunteers are listed in Table 1. The conditions to become a volunteer in this study were as follows:

- Nonsmoker
- Male
- No use of coal tar-containing shampoo/soap or ointment
- No occupation with recognized or expected PAH exposure
- No treatment for skin disease.

The volunteers played a match on a football ground that has been filled with recycled tire crumb infill 2 months before. The match lasted 90 min with a break of 15 min. Before playing the match, training with ground exercises for 30 min was done. This was done to mimic heavy dermal contact, with sliding, sitting and crawling over the ground. This was followed by a warming-up of 15 min. The total exposure period lasted 2.5 h.

Table 1 Characteristics of volunteers that played football on an artificial playing ground with recycled rubber infill

Person	Age (years)	Weight (kg)	Profession	Use of medicines? (yes/no)
A[a]	26	67	Teacher	No
B[a]	24	70	Sales manager	No
C	20	74	Telecom-consultant	No
D[a]	23	85	Fish dealer	Yes, Seretide (asthma)
E	21	104	Salesman electrical equipment	No
F	21	76	Student	No
G	31	72	Junior psychiatrist	No

[a] Persons A, B and D underwent a massage of the legs prior to the exercises on the playground with Chemodol®

Concomitant exposure to PAH from the diet, environmental tobacco smoke (ETS) and outdoor air sources such as diesel engine emissions (DEE) and barbeque smoke (BBQ) can alter the baseline elimination and may confound the results of this experimental test. In order to minimize the confounding, the volunteers were instructed as

- not to eat black cured meat: no barbecue, no spare-ribs and no fried bacon,
- not to eat smoked meat and/or fish,
- not to use open fire in the house,
- not to use PAH-containing hobby/do it yourself products such as tar, carbolineum, bitumen,
- not to drink more than two glasses of beer/wine of liquor per day,
- to minimize the stay in rooms with environmental tobacco smoke,
- not to use stimulating drugs like marihuana, paddo's or so-called party drugs, during the 3 day-sampling period.

Three volunteers (A, B and D) underwent a massage with massage oil, Chemodol®. In advance of the warming-up, training exercises and match, the three volunteers had a massage of the legs with massage oil, Chemodol®. This Chemodol oil is often used by football players.

During a period of 3 days, all urine voidings of the volunteers were sampled. Sampling was started a day before the experiments in order to assess the individual background elimination of each volunteer. Each volunteer was instructed to record the time and volume of each urine voiding. A 100 ml sample of the voiding was immediately stored in the freezer at −18°C. The sporting activities with possible exposure were on the second day.

The sampling started in the morning on the day before the exposure experiment and lasted till the evening of the day after the exposure experiment.

In order to help remember the volunteers of their duties and to get a regular voiding over time each 2.5 h a SMS message was sent to each volunteer. In average, a total of 20 samples per volunteer were collected over the 3-day study period.

Concentration of PAH in the rubber crumb infill

Total PAH components are found in the rubber infill in amounts of 20–40 mg/kg. A sample of the rubber crumb infill from the football playing ground was taken and analyzed for PAH. PAH were ultrasonically extracted with dichloromethane. After centrifugation, the extract was analyzed for PAH by high-performance liquid chromatography and fluorescence detection using multiple wavelength shift for simultaneous quantification. The total PAH concentration was 24 mg/kg (Hofstra, 2007). The crumb infill size was measured as <2 mm.

Determination of hydroxypyrene in urine

Following collection, urine specimens were labeled, frozen and shipped to the laboratory (AML Laboratories, Antwerpen, Belgium) for 1-hydroxypyrene analyses according to the method of Jongeneelen (Jongeneelen et al. 1987). In short: the total of the free and conjugated 1-hydroxypyrene in urine was determined with high-pressure liquid chromatography (HPLC). After enzymatic hydrolysis, to release the conjugated part of 1-hydroxypyrene, the analyte is separated from the matrix and analyzed by HPLC with a fluorescence detector.

The concentration of 1-hydroxypyrene is adjusted to creatinine excretion and expressed as μmol/mol creatinine. The detection limit of 1-hydroxypyrene in urine was 0.46 nmol/L.

Quality control of the analyses

For purposes of quality assurance and control, AML participated in the German DFG program of round-robin proficiency testing for urinary 1-hydroxypyrene.

A series of 10 duple samples were analyzed to test the accuracy of the analysis. The coefficient of variation of the analysis of creatinine and 1-hydroxypyrene was 0.8 and 10.6%, respectively.

Undetectables, very diluted and very concentrated samples

Highly diluted or concentrated urine samples may lead to erroneous results due to altered excretion mechanisms. Samples with a creatinine concentration beyond the range of 4.4–26.5 mmol/L were treated as out of range and were excluded from the data set as recommended by ACGIH. In total, 25 of 138 samples were excluded. Eight out of the remaining 113 samples had a concentration of 1-hydroxypyrene below the detection limit. These samples were processed as having the value of two-thirds of the detection limit.

Results

The football players spent in total 2.5 h on the artificial grass field. They performed training activities with intensive skin contact, did a warming-up and played a football match directly afterward. Visual inspection of the skin of the football players after this period showed that the players had a black residue of crumb dust on knees, hand

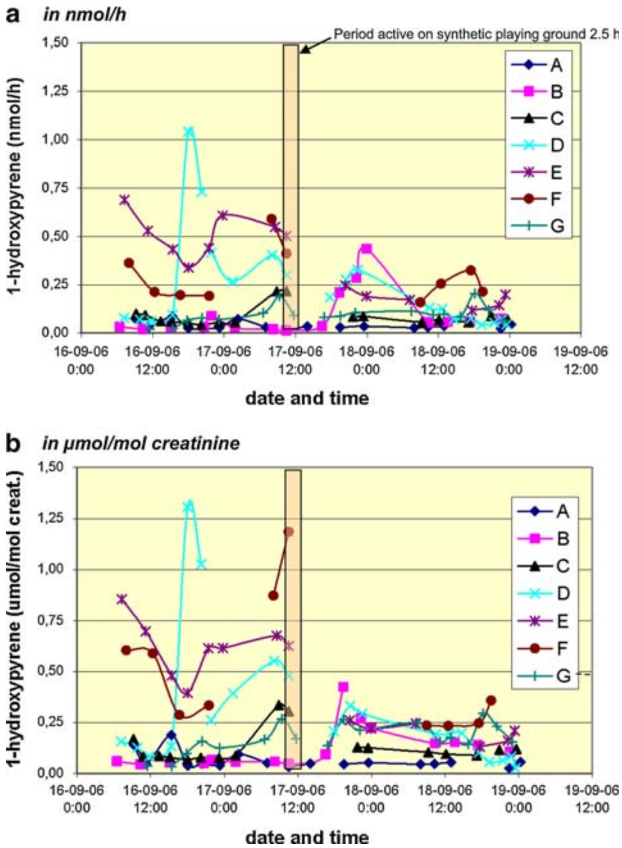

Fig. 1 Elimination of 1-hydroxypyrene in urine of seven volunteers before, during and after, the 2.5-h lasting exercises and football match on an artificial football ground with rubber crumb infill **a** in nmol/h, **b** in µmol/mol creatinine

palms and elbows. This observation confirmed that skin contact had occurred to dust of the tire crumb.

Figure 1 shows the amount of 1-hydroxypyrene elimination over time of all seven volunteers in nmol/h (a) and in µmol/mol creatinine (b).

The period before the sporting period gives the individual baseline elimination rate of 1-hydroxypyrene in urine. Table 2 presents the individual pre-sporting elimination rate of hydroxypyrene and the post-sporting elimination rate. It shows that 3 out of 7 volunteers (persons D, E and F) had relatively high and variable baseline elimination rate. The baseline of the other 4 volunteers (persons A, B, C and G) is lower. Only one volunteer (person B) showed a significant increase of the elimination after the football match.

Three volunteers (A, B and D) underwent in advance of the sporting activities a massage with massage oil Chemodol®. There was no difference in hydroxypyrene excretion compared with volunteers without the Chemodol treatment. No enhancing or suppressing effect on the skin absorption of pyrene could be detected.

Discussion

An experimental field study was performed. Urinary excretion was determined before and after sporting on the synthetic play ground. The half-life of urinary hydroxypyrene is estimated between 5 and 20 h (ACGIH 2005; WHO 1996; Viau et al. 1995). That means that a sampling period of 3 days is sufficient long to estimate the individual baseline level before the sporting activities and to record an increase of the baseline level due to exposure to or contact with the rubber infill.

Dietary intake of PAH and smoking are the main source of environmental exposure to PAH and 1-hydroxypyrene in urine. The committee Human Biomonitoring of the German Federal Environmental Agency (Umweltbundes Ambt) has proposed a reference value of 1-hydroxypyrene for non-smokers: 0.5 µg/L = 0.3 µg/g creatinine = 0.16 µmol/mol (FEA (Federal Environmental Agency of Germany) 2005. This level is based on the observations in people without occupational or significant environmental exposure. Levels of 1-hydroxypyrene vary from country to country, probably due to variations in the environmental PAH background and/or dietary intake of PAH. In the Netherlands, the average of 1-hydroxypyrene concentration in urine of young adult nonsmokers is estimated to be 0.12 (95% CI = 0.04–0.29) µmol/mol creatinine (van Rooij et al. 1994). Assuming a creatinine excretion of 15.9 mmol/24 h,

Table 2 Pre-sporting and post-sporting urinary elimination rate of 1-hydroxypyrene in football players

Person	Pre-sporting excretion AM (SD) nmol/h	Post-sporting excretion AM (SD) nmol/h	Difference between post- and pre-sporting	Statistical significant increase
A	0.04 (0.03)	0.03 (0.01)	−0.01	–
B	0.03 (0.02)	0.14 (0.13)	+ 0.11	$P = 0.004$
C	0.10 (0.07)	0.07 (0.01)	−0.03	–
D	0.35 (0.34)	0.15 (0.11)	−0.20	–
E	0.48 (0.14)	0.20 (0.07)	−0.24	–
F	0.33 (0.16)	0.24 (0.07)	−0.09	–
G	0.08 (0.05)	0.11 (0.04)	+0.03	–

the average is estimated to be equal to 0.08 nmol/h (95% confidence interval: 0.03–0.19).

Figure 1 shows that the pre-sporting levels of 1-hydroxypyrene of persons D, E and F were already increased compared with the Netherlands reference values. These three volunteers were interviewed afterward on their activities with possible exposure from dietary and environmental PAH. No full explanation was found, but it appeared that person D might have been exposed to PAH from smoke condensate in the fish shop, person E might have been exposed to PAH in an electrical equipment store and person F might have been exposed to PAH from environmental tobacco smoke during his evening work in a pub. The unforeseen pre-sporting exposure of the persons D, E and F to PAH from other sources reduces the sensitivity to detect PAH-exposure due to sporting in the experimental period. The data of these three persons were omitted for further conclusions on exposure due to sporting on an artificial ground.

The 1-hydroxypyrene levels in the pre-sporting period of the remaining four volunteers (person A, B, C and G) were normal, and the intraindividual variation was small. One of these four volunteers (person B) showed an increased excretion after sporting. However, interviews afterward made clear that person B had consumed a fried hamburger directly after the sporting activities. This coincides with the possible exposure by sporting on the artificial ground and may be as well the reason of the increased excretion of 1-hydroxypyrene. Also, his friend person D ate a fried hamburger at the same moment, and his excretion profile showed also some enhancement. Buckley and Lioy (1992) reported that mean elimination rates during minimal dietary exposure periods ranged from 6 to 17 ng/h (=0.03–0.08 nmol/h), whereas peak elimination rates of 60–189 ng/h (=0.28–0.87 nmol/h) were seen after a meal high in PAH. This confirms the possible confounding by dietary intake of PAH.

Data on 1-hydroxypyrene excretion during several consecutive days in nonoccupationally exposed persons are hardly available or published. This study shows that the interindividual and intraindividual variation of background 1-hydroxypyrene levels can be quite high, despite considerable efforts to limit this variation. This finding indicates that there are sources of PAH-exposure that are not yet identified (e.g., possible sources in a store for electrical consumer products, such as TVs, radios, washing machines, stoves) or that appear more relevant than expected (e.g., the consumption of a hamburger).

The strong and unexpected variation in background 1-hydroxypyrene levels of the volunteers complicates the comparison of the PAH-exposure before and after the exposure to rubber infill for each individual. But if we also compare the urinary 1-hydroxypyrene levels of the football players, that were measured after being intensively exposed to rubber infill, with the levels measured in a reference group of young adult nonsmokers in the Netherlands, than the results show that the PAH uptake from rubber crumb infill is of no major concern.

Conclusion

This study provides evidence that uptake of PAH of football players active on artificial grass fields with rubber crumb infill is minimal. If there is any exposure, then the uptake is very limited and within the range of uptake of PAH from environmental sources and/or diet.

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