



HAL
open science

Urinary elimination kinetics of 3-hydroxybenzo(a)pyrene and 1-hydroxypyrene of workers in a prebake aluminum electrode production plant: Evaluation of diuresis correction methods for routine biological monitoring

Simon Lutier, Anne Maitre, Vincent Bonneterre, Dominique J. Bicout, Marie Marques, Renaud Persoons, Damien Barbeau

► To cite this version:

Simon Lutier, Anne Maitre, Vincent Bonneterre, Dominique J. Bicout, Marie Marques, et al.. Urinary elimination kinetics of 3-hydroxybenzo(a)pyrene and 1-hydroxypyrene of workers in a prebake aluminum electrode production plant: Evaluation of diuresis correction methods for routine biological monitoring. *Environmental Research*, 2016, 147, pp.469-479. 10.1016/j.envres.2016.02.035 . hal-01904513

HAL Id: hal-01904513

<https://hal.science/hal-01904513>

Submitted on 19 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Urinary elimination kinetics of 3-hydroxybenzo(a)pyrene and 1-hydroxypyrene of workers in a prebake aluminum electrode production plant: Evaluation of diuresis correction methods for routine biological monitoring

Simon Lutier^a, Anne Maître^{a,b}, Vincent Bonneterre^{a,b}, Dominique Bicout^a,
Marie Marques^a, Renaud Persoons^b, Damien Barbeau^{a,b}

^aEPSP-TIMC (CNRS UMR 5525), Université Grenoble Alpes, TIMC-IMAG, F-38000 Grenoble, France

^bLaboratoire de Toxicologie Professionnelle et Environnementale, DBTP, CHU de Grenoble, France

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous carcinogenic pollutants emitted in complex mixtures in the ambient air and contribute to the incidence of human cancers. Taking into account all absorption routes, biomonitoring is more relevant than atmospheric measurements to health risk assessment, but knowledge about how to use biomarkers is essential. In this work, urinary elimination kinetic of 1-hydroxypyrene (1-OHP) and 3-hydroxybenzo(a)pyrene (3-OHBP) were studied in six electrometallurgy workers after PAHs exposure. Spot samples were collected on pre- and post-shift of the last workday then the whole urinations were separately sampled during the weekend. Non-linear mixed effects models were built to study inter- and intra-individual variability of both urinary meta-bolites toxicokinetic and investigate diuresis correction ways. Comparison of models confirmed the diuresis correction requirement to perform urinary biomonitoring of pyrene and BaP exposure. Urinary creatinine was found as a better way than specific gravity to normalize urinary concentrations of 1-OHP and as a good compromise for 3-OHBP. Maximum observed levels were 1.0 $\mu\text{mol/mol}$ creatinine and 0.8 nmol/mol creatinine for 1-OHP and 3-OHBP, respectively. Urinary 1-OHP concentrations on post-shift were higher than pre-shift for each subject, while 3-OHBP levels were steady or decreased, and maximum urinary excretion rates of 3-OHBP was delayed compared to 1-OHP. These results were consistent with the sampling time previously proposed for 3-OHBP analysis, the next morning after exposure. Apparent urinary half-life of 1-OHP and 3-OHBP ranged from 12.0 h to 18.2 h and from 4.8 h to 49.5 h, respectively. Finally, inter-individual variability of 1-OHP half-life seemed linked with the cutaneous absorption extent during exposure, while calculation of 3-OHBP half-life required the awareness of individual urinary background level. The toxicokinetic modeling described here is an efficient tool which could be used to describe elimination kinetic and determine diuresis correction way for any other urinary biomarkers of chemicals or metals exposure.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants emitted during incomplete combustion of organic matter

and distillation of coal or petroleum. They are found in the atmosphere as complex gaseous and particulate mixtures, whose composition varies according to the emission sources. Within this chemical family, benzo(a)pyrene (BaP) is classified as a proven human carcinogen while several other PAHs are classified as possible or probable human carcinogens (IARC, 2010). By contrast, pyrene is a non-carcinogenic PAH, extensively studied due to its abundance in mixtures. Environmental exposure occurs by tobacco consumption, diet, and atmospheric pollution. In addition, high occupational exposure levels are reported in some industries using products derived from coal (Unwin et al., 2006). In France,

Abbreviations: PAHs, Polycyclic Aromatic Hydrocarbons; 3-OHBP, 3-hydroxybenzo(a)pyrene; 1-OHP, 1-hydroxypyrene; SG, Urinary specific gravity

* Correspondence to: Equipe Environnement et Prédiction de la Santé des Populations, Laboratoire TIMC-IMAG, UMR CNRS 5525, Université Joseph Fourier – Grenoble 1, UFR de Médecine, Domaine de la Merci, 38700 La Tronche, France.

E-mail address: DBarbeau@chu-grenoble.fr (D. Barbeau).

1.6 million workers are exposed to PAHs with 93,000 worked in the metallurgy industry (Guignon and Sandret, 2005) including carbon electrode manufacturing, which is classified as probably carcinogenic to humans (IARC, 2010). In this sector, the mean levels reported for BaP and pyrene were $4.9 \mu\text{g}/\text{m}^3$ and $11.2 \mu\text{g}/\text{m}^3$, respectively (Forster et al., 2008). These levels were largely higher than the French recommended value for BaP, equal to $150 \text{ ng}/\text{m}^3$. However, atmospheric measurement cannot assess human exposure: wearing respiratory mask can generate an over-estimation of exposure and dermal uptake, which is the main absorption way of PAHs for numerous tasks, can lead to an under-estimation of exposure (Elovaara et al., 1995; VanRooij et al., 1993).

For assessing health risk, biomonitoring is more relevant than air monitoring because it allows the evaluation of the internal dose of chemicals. Urinary analyses are widely used, because exposure biomarkers accumulate in the bladder between two urinations and it is very easy to collect urine samples. To take into account the variation of diuresis, urinary concentrations stated in mass per volume unit need to be corrected. Normalization by urinary creatinine is mainly used although age, sex, ethnicity and muscular mass influence its excretion and can change the interpretation of urinary biomarkers levels (Truchon et al., 2014). Furthermore, this correction is valuable only if metabolites undergo the same excretion mechanisms than the creatinine, *ie* via glomerular filtration (Boeniger et al., 1993). To overcome these difficulties, normalization by specific gravity (SG) has been proposed to take into account fluctuations in urine dilution (Sauvéet et al., 2015). Nowadays, research on the optimal approach to adjustment of urinary concentrations in spot urine samples, in order to perform biomonitoring, is still an important challenge (Weaver et al., 2015).

Urinary 1-hydroxypyrene (1-OHP), the main metabolite of pyrene, was proposed as the first biomarker of PAHs exposure by Jongeneelen in 1985 (Jongeneelen et al., 1985). It is the most commonly used biomarker to perform PAH biomonitoring (Strickland et al., 1996) and many exposure data are available (Hansen et al., 2008). 1-OHP was correlated with genotoxic effect in some studies (Sivińska et al., 2004; Talaska et al., 2014), but not systematically (Marczynski et al., 2011), probably due to the high variability of the BaP/pyrene ratio according to industrial sectors and activities of workers (Barbeau et al., 2015; Lafontaine et al., 2004). Thus, 1-OHP might be not the most appropriate biomarker to assess carcinogenic PAHs exposure. Urinary 3-hydroxybenzo(*a*) pyrene (3-OHBP) is more appropriate to evaluate carcinogenic exposure because it is an urinary metabolite of BaP (Boogaard, 2008). Due to a complex BaP metabolism with production of many metabolites (Gelboin, 1980) and elimination predominantly via the feces, urinary 3-OHBP represented only 0.21% of BaP dose injected in rats (Marie et al., 2010). A high sensitive and reliable analytical method has recently been developed to use urinary 3-OHBP as biomarker for occupational biomonitoring (Barbeau et al., 2011). The maximum urinary 3-OHBP concentration of workers involved in an electrode manufacturing plant was found at $5.27 \text{ nmol}/\text{mol}$ creatinine, while maximum of 1-OHP was higher by three orders of magnitude, reaching $6.99 \mu\text{mol}/\text{mol}$ creatinine (Barbeau et al., 2014).

In order to set the best sampling time representing the exposure and being convenient to implement, biomonitoring requires knowledge about elimination kinetic and related influencing factors. The different absorption routes affect considerably the urinary elimination rate of 1-OHP. In human, the maximum urinary excretion took place between 2.2 h and 9.7 h after a dietary exposure (Buckley and Lioy, 1992; Chien and Yeh, 2010; Li et al., 2012), while this maximum appeared 1–2 h after the post-shift for a respiratory absorption (Lafontaine et al., 2002) and 10–15 h for a dermal uptake (Viau and Vyskocil, 1995). In the same way, the

urinary half-life of 1-OHP ranged from 3.0 to 5.7 h (Li et al., 2012), from 3.7 to 9.9 h (St Helen et al., 2012) and from 11.5 to 15 h (Viau and Vyskocil, 1995) after oral, inhalation and dermal absorption, respectively. Thus, in addition to individual characteristics, differences of exposure conditions in workplaces, especially the cutaneous absorption extent, could explain the important variability of apparent 1-OHP half-lives observed in occupational studies, as well in different industrial sectors (Lafontaine et al., 2004), as in the same sector for several tasks (Buchet et al., 1992; Sobus et al., 2009). Pre-shift urine sample collected at the end of the workweek is considered as the best sampling time to analyse 1-OHP when the main route of exposure is skin uptake, while post-shift sample collected at the end of the workweek remains the best if inhalation is the main way of absorption (Bouchard and Viau, 1999). However, there is still no consensus and ACGIH recommends only post-shift at end of workweek as sampling time for 1-OHP (ACGIH, 2015). For 3-OHBP, maximum urinary excretion appeared between 10 and 17 h after the post-shift (Gendre et al., 2002) and the only apparent urinary half-life calculated in humans for this metabolite was 8.8 h (Lafontaine et al., 2004). But urinary 3-OHBP concentrations measured in the urine collected at the post-shift of the penultimate workday were not statistically different than those found at the pre-shift of the last workday, when the exposure is relatively constant over the workweek (Barbeau et al., 2014). Conversely, when occupational exposure is variable during the workweek, 60% of the maximum urinary 3-OHBP concentrations were found on pre-shift, versus 17% on post-shift of the last workday (Barbeau et al., 2015). Currently, pre-shifts of the first and of the last workdays remain the proposed urine sampling times to analyse this metabolite (Gendre et al., 2004).

The aim of this study was to fill the lack of data about the toxicokinetic of urinary 3-OHBP and make a comparison with 1-OHP, the common PAH exposure biomarker. Furthermore, the usefulness of different kinds of diuresis correction was studied for these urinary metabolites. The maximum urinary excretion rate, the influence of inter-individual variability and the appropriate normalization technique of urinary concentrations were studied using non-linear mixed effects models in a group of electro-metallurgy workers.

2. Materials and methods

2.1. Study population

The subjects were 6 non-smoking, healthy, male volunteers between 30 and 60 years old, working in a prebaked electrodes production plant. A questionnaire was completed by each worker in order to inform them about the study and obtain their consent. No special instruction concerning diet was given. Urinary analyses were prescribed by the occupational physician of the plant as part of PAHs exposure monitoring. During the last day of the workweek, one worker (W1) stored the cooled electrode after its extrusion, three workers (W2, W3, W4) loaded raw materials (carbon and pitch coke), one (W5) was in charge of administrative tasks in an office and the last one (W6) performed electrode extrusion and cleaning installations. All workers carried P3 masks and handling gloves, except workers 5 and 6.

2.2. Urine collection

Urine samples were collected at the beginning and at the post-shift on the last workday of the week in 50 ml propylene bottles. From this post-shift, subjects collected separately all urinations in their entirety in 500 ml propylene bottles during the weekend. Urine volume was measured at the laboratory before storage at

Table 1
Urinary 1-OHP and 3-OHBP concentrations.

Metabolite	Worker	Creatinine adjustment (nmol/mol creatinine)			SG adjustment (pmol/l)			Non adjusted concentration (pmol/l)			N (n < LOQ)
		Pre-shift	Post-shift	Week-end (min-max)	Pre-shift	Post-shift	Week-end (min-max)	Pre-shift	Post-shift	Week-end (min-max)	
1-OHP	W1	420	500	30–660	5703	5303	367–8251	7514	6644	477–7885	13 (0)
	W2	320	580	60–690	4708	9875	665–10,381	4948	12,005	916–11,088	14 (0)
	W3	530	700	130–1000	7010	9311	2192–15,887	4948	11,867	2712–20,871	11 (0)
	W4	690	860	50–700	5876	8322	454–8156	5911	5727	545–6460	19 (0)
	W5	580	730	260–750	7894	10,830	2123–8455	11,455	17,136	820–11,721	8 (0)
	W6	310	400	120–250	2286	3991	944–2512	1649	4765	527– 3556	10 (0)
3-OHBP	W1	0.13	0.16	< LOQ–0.26	1.70	1.73	< LOQ–3.31	2.24	2.16	< LOQ–2.78	13 (2)
	W2	0.40	0.23	0.02–0.39	5.85	3.86	0.22–5.06	6.15	4.70	0.34–7.78	14 (0)
	W3	0.04	0.05	0.02–0.03	0.53	0.70	0.26–0.43	0.37	0.89	0.28–0.54	11 (0)
	W4	0.80	0.64	0.07–0.28	6.85	6.23	0.55–3.20	6.90	4.29	0.50–2.54	19 (0)
	W5	0.18	0.16	0.17–0.27	2.47	2.38	1.45–2.62	3.58	3.76	0.56–3.63	8 (0)
	W6	0.19	0.12	0.02–0.11	1.45	1.22	0.16–1.21	1.04	1.45	0.09–1.24	10 (0)

LOQ: limit of quantification.

–20 °C until analysis. Data of W6 was excluded for toxicokinetic modeling because urine samples were not collected during the first 24 h following post-shift.

2.3. Urinary analyses

After thawing urine overnight, the 3-OHBP analysis was conducted using the method previously described by Barbeau et al. (2011). Briefly, a 10 ml aliquot was diluted in acetate buffer before enzymatic hydrolysis. The sample was purified and concentrated by automated solid phase extraction from Gilson (Villiers-le-Bel, France) using Sep-Pak C18, 500 mg with a capacity of 6 ml from Waters (Guyancourt, France). Urinary 3-OHBP was quantified by high pressure liquid chromatography coupled with fluorescence detection (HPLC-FLD). The heart-cut technique (Campins-Falco et al., 1993) was used: a first chromatographic separation was performed on a purification Supelcosil LC-18 column (50 mm × 4 mm, 5 µm) from Sigma-Aldrich (Saint-Quentin-Fallavier, France), then a second on an analytical column PAH-C18 (250 mm × 3 mm, 5 µm) from Waters. Excitation and emission wavelengths were fixed at 425 and 465 nm, respectively. The limit of quantification (LOQ) was 0.2 pmol/l (0.05 ng/l).

Urinary 1-OHP was determined using a modified method of that previously described by Jongeneelen et al. (1988). Briefly, a 2 ml aliquot of urine was diluted in acetate buffer before enzymatic hydrolysis and directly injected into a HPLC-FLD system from Waters. On-line extraction of 1-OHP was performed with a Supelguard C18 column from Sigma-Aldrich and elution was performed with a LiChrospher C18 column from Merck (Billerica, USA). Excitation and emission wavelengths were fixed at 333 and 390 nm, respectively. The LOQ was 91.6 pmol/l (20 ng/l). The laboratory meets the German Society for Occupational and Environmental Medicine external quality control system standards for 1-OHP.

Urinary creatinine was determined by Jaffe's method from a 2 ml aliquot of urine, while urinary SG was measured using a refractometer PEN-Urine S.G from ATAGO (Tokyo, Japan). For each sample, three successive measurements of SG were performed and arithmetic means was calculated. SG of distilled water (typically equal to 1) was measured at regular time interval to avoid any drift across time.

2.4. Data analyses

Metabolites concentrations lower than the LOQ were assigned values equal to half of the LOQ for graphical representations and data analyses.

Metabolites excretion rates (mol/h) were calculated from the first urine after the post-shift, by the sum of metabolites quantities excreted in different urine collected during five periods of 12 h. These quantities were obtained by multiplying concentration by volume for each sample.

Urinary metabolite concentrations were normalized by urinary creatinine (mol/mol creatinine), or by SG (mol/l) as follow:

$$C_{Cr}(\text{mol/mol creatinine}) = \frac{C}{Cr} \quad (1)$$

$$C_{SG}(\text{mol/l}) = \frac{C \times (SG_{ref} - 1)}{(SG - 1)} \quad (2)$$

where C was uncorrected metabolite urinary concentration (mol/l), Cr was urinary creatinine concentration (mol/l), SG was urinary specific gravity of the sample and $SG_{ref} = 1.017$ was the reference specific gravity, i.e. the calculated mean of SG values from all collected urines.

2.5. Toxicokinetic modeling

3-OHBP and 1-OHP elimination kinetics were studied from the observed individual excretion maxima that is the urinary sample after occupational exposure for which creatinine adjusted concentration of metabolites was the highest. To describe these data, a non-linear mixed effect model which assumed a mono-exponential urinary elimination kinetic and takes into account background exposure and inter-individual variability was used (Bartell, 2012). In order to compare different correction modes of diuresis, three models were developed from unadjusted urinary concentrations. Firstly, urinary creatinine concentration was included as independent variable, according to Eq. (3) (Cr-model):

$$C_{ij} = (Cm_i \times e^{-k_i \times t_{ij}} + CO_i) \times Cr_{ij} + \epsilon_{ij} \quad (3)$$

where i was an index for workers and j was an index for each urinary sample. t_{ij} was the time from individual excretion maximum (h). C_{ij} was metabolite concentration (mol/l) and Cr_{ij} was

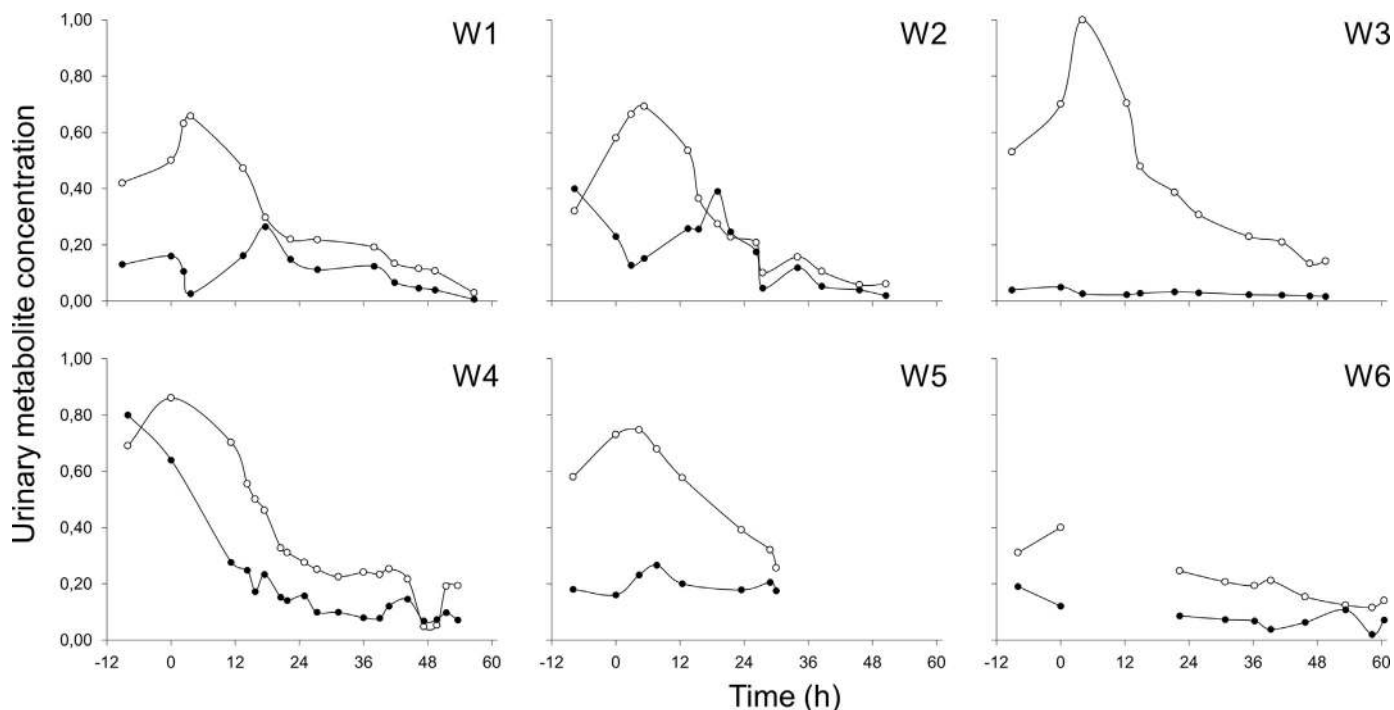


Fig. 1. Urinary creatinine adjusted concentration of 1-OHP ($\mu\text{mol/mol}$ creatinine, open circle) and 3-OHbAP (nmol/mol creatinine, closed circle) during the weekend of 6 electrometallurgy workers (W). Sample at post-shift of the last workday took place at time 0 and the previous spot urine sample is the pre-shift of the same day.

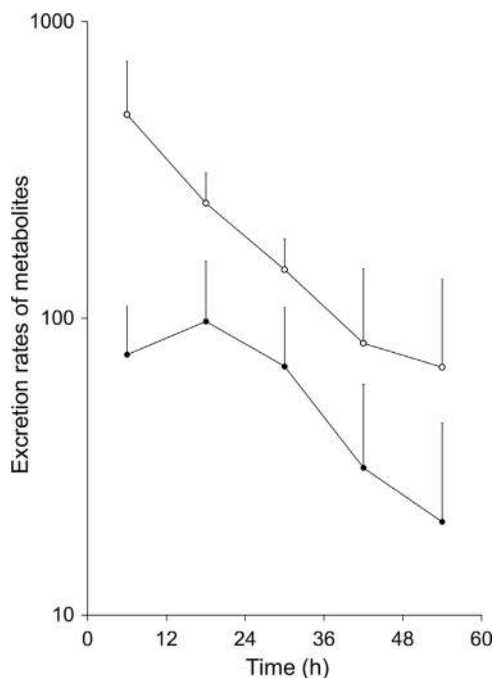


Fig. 2. Average and standard deviation of excretion rates into twelve hours periods for 1-OHP (pmol/h; open circles) and 3-OHbAP (fmol/h; closed circles).

creatinine concentration (mol/l), for urine j of worker i . Cm_i (mol/mol creatinine) was the concentration increase above the background after occupational exposure. CO_i (mol/mol creatinine) was the background concentration. k_i was the individual first-order urinary elimination rate constant (1/h) and ε_{ij} was the error term.

Secondly, urinary SG was included as independent variable instead of creatinine, according to Eq. (4) (SG-model):

$$C_{ij} = (Cm_i \times e^{-k_i \times t_{ij}} + CO_i) \times \frac{SG_{ij}-1}{SG_{ref}-1} + \varepsilon_{ij} \quad (4)$$

Finally, no correction term was introduced as Eq. (5) (Zero-model):

$$C_{ij} = Cm_i \times e^{-k_i \times t_{ij}} + CO_i + \varepsilon_{ij} \quad (5)$$

For SG-model and Zero-model, Cm_i and CO_i were expressed in mol/l.

For the three models, CO_i , Cm_i and k_i were further assumed to be independent and normally distributed among workers, with mean μCO , μCm and μk , respectively. Akaike Information Criterion (AIC) was used for models comparison and the percentage of root mean square error (%RMSE) was used to compare their residual error.

Apparent urinary half-life ($t_{1/2}$) was calculated as Eq.6:

$$t_{1/2} = \frac{\ln 2}{k} \quad (6)$$

Non-linear mixed effect models were performed with R 3.1.2 software (R Foundation for Statistical Computing) using "nlme" function from "nlme" package (Pinheiro and Bates, 2000). Parameters were maintained in the models if they were significantly different from null at $p \leq 0.10$.

3. Results

3.1. Urinary levels

Urinary levels of both metabolites for the last workday and during the weekend were summarized in Table 1 and showed in Fig. 1. Urinary 1-OHP concentrations were systematically higher at post-shift than pre-shift. For 3-OHbAP, concentrations were steady between pre- and post-shift for 3 workers (W1, W3 and W5) and decreased for the others (W2, W4 and W6). Among 65 urine samples, only 2 concentrations were below the LOQ for 3-OHbAP (W1) and none for 1-OHP. From the end of the shift and after exclusion of W6, urine samples were collected during 53 ± 3 h except for W5 who stopped sample collection after 30 h. For

1-OHP, average of minimum concentrations and average of maximum concentrations were 108 and 727 nmol/mol creatinine, 1124 and 9610 pmol/l and 1000 and 11,520 pmol/l, for creatinine adjusted, SG adjusted and non-adjusted concentrations, respectively. For 3-OHBP, they ranged from 0.05 to 0.33 nmol/mol creatinine, 0.45–3.46 pmol/l and 0.30–3.93 pmol/l. Among workers, urinary 1-OHP levels were less fluctuating than 3-OHBP, with relative standard deviation of maximum concentrations equal to 28% versus 79% for creatinine adjusted, 41% versus 70% for SG adjusted and 55% versus 72% for non-adjusted concentrations.

3.2. Urinary elimination rates

Urinary elimination rates profiles were different between the two metabolites during the first 24 h after the shift (Fig. 2). For 1-OHP, mean excretion rates decreased constantly throughout sampling time period. Conversely, for 3-OHBP, mean excretion rates increased slightly from 75 to 98 fmol/h between 0–12 h and 12–24 h periods, before decreasing until the end of urine sampling.

3.3. Elimination kinetic models

Figs. 3–7 showed the observed and predicted urinary 1-OHP and 3-OHBP concentrations for each worker. Models including correction term (Cr-model and SG-model) improved the model fitting for both metabolites compared to the Zero-model. Normalized residuals of predicted urinary 1-OHP and 3-OHBP concentrations were heteroscedastic from Zero-model whereas those from Cr-model and SG-model were more homoscedastic (Fig. A.1). Scatter plots of observed versus predicted urinary concentrations indicated a better fit for models included diuresis correction term (Cr-model and SG-model) than Zero-model (Fig. A.2). Akaike Information Criterion (AIC) for Cr-model, SG-model and Zero-model were –600.38, –555.97 and –497.40, respectively, for 1-OHP and –581.53, –581.25 and –567.09 for 3-OHBP. Residual variance of each model, compared with %RMSE, decreased in order Zero-model (48%) > SG-model (28%) > Cr-model (18%) and Zero-model (43%) > SG-model (32%) ≈ Cr-model (31%), for 1-OHP and 3-OHBP, respectively (Fig. 8). Calculated parameters for Cr-model and both metabolites were summarized in Table 2. μ_{Cm} and μ_k were significantly different from null, for both metabolites, while μ_{CO} was not significantly different from null for 1-OHP. Mean calculated urinary 3-OHBP half-life was lower than 1-OHP with 8.9 h and 14.4 h respectively. Inter-individual variability was higher for 3-OHBP than for 1-OHP with calculated half-life confidence interval wider.

4. Discussion

Elimination kinetics of urinary PAHs exposure biomarkers was studied for six workers after a whole workweek. Maximum observed levels adjusted with creatinine were 1.0 $\mu\text{mol/mol}$ creatinine and 0.8 nmol/mol creatinine for 1-OHP and 3-OHBP, respectively. These values are lower than those previously reported in the same industrial sector (Petry et al., 1996) but was in consistency with decreasing of levels over the last 20 years. Average of minimum observed concentrations adjusted with creatinine was 0.11 $\mu\text{mol/mol}$ creatinine and 0.06 nmol/mol creatinine for 1-OHP and 3-OHBP, respectively. For both metabolites, the highest minimum concentrations were found for W5 because he collected urines only for the thirty first hours after post-shift. Such urinary levels at the end of the weekend in non-smoking workers corresponded to those of non-occupationally exposed smokers which ranged from 0.03 to 0.53 $\mu\text{mol/mol}$ creatinine and from < 0.01 to

0.08 nmol/mol creatinine for 1-OHP and 3-OHBP, respectively (Barbeau et al., 2011; Lafontaine et al., 2006). Increase risk of cancer due to smoking is well-known and PAHs belong to carcinogenic compounds emitted by tobacco combustion (IARC, 2004). Thus, even for moderate occupational PAHs exposure noticed here, the health risk for workers is real.

Excretion rate profiles of the two urinary metabolites were clearly different. While 1-OHP followed a mono-exponential elimination as soon as the end of the shift, 3-OHBP mean excretion rates slightly increased during the first 24 h, probably due to an accumulation of 3-OHBP in the kidney as shown in rats (Marie et al., 2010). Indeed, physiologically-based pharmacokinetic model developed from rats data was used to modeling human 3-OHBP urinary elimination kinetic and the results seemed indicate that specific 3-OHBP kinetic, including atypical renal excretion, is similar between human and rat (Heredia Ortiz et al., 2014). Among workers involved in different industrial sectors, an important inter-individual variability of excretion gap between the two metabolites was shown, with a delay for 3-OHBP ranging from 3 to 24 h (Gendre et al., 2004). In case of occupational exposure very variable one day to another, 60% of urinary 3-OHBP maximum were occurred at the pre-shift of the last workday (Barbeau et al., 2015). On the other hand, in case of steady occupational exposure throughout workweek, urinary 3-OHBP concentrations were not statistically different between sample at post-shift of penultimate workday and sample at pre-shift of the following (Barbeau et al., 2014). These discrepancies complicate the choice of an appropriate sampling time for urinary 3-OHBP. Urinary metabolites excretion rates were calculated to reduce the error on the assessment of maximum 3-OHBP excretion that could come from the use of urinary concentrations (mass per volume unit). Such a method is more relevant because it takes into account the total excreted quantities in function of time. The urinary excretion delay between 3-OHBP and 1-OHP, observed even from excretion rates, confirmed that urinary 3-OHBP analysis should be performed the next morning after occupational exposure rather than at post-shift.

Considering workers as a random effect on urinary biomarkers concentrations, kinetic models built in this study were able to estimated inter-individual variability of PAHs uptake (C_m), urinary background (CO) and toxicokinetic process (k) coming from occupational exposure conditions, lifestyle and personal characteristics, respectively. By contrast, intra-individual variability participated to residual variance (variance of error term). If diuresis was a factor of intra-individual variability of urinary PAHs metabolites concentrations, addition of a term linked to urine dilution in the models could reduce residual variance and improve the parameters calculation. Indeed, normalized residuals of Zero-model were heteroscedastic for both metabolites, while addition of a variable adjusting urine dilution allowed decreasing of heteroscedasticity. In the same way, scatter plots indicated a better fit for models included diuresis correction. Finally, AICs which penalize complex models were lower for Cr-model and SG-model even if they included one more independent variable than Zero-model. These results confirmed the diuresis correction requirement to perform urinary biomonitoring of pyrene and BaP exposure. Previous mixed effects models simulating urinary elimination kinetic of gaseous PAH metabolites after dietary exposure were developed from urinary creatinine corrected concentrations (Li et al., 2012; Motorykin et al., 2015). This approach assumed that urinary creatinine correction is a relevant normalization method while age, sex, ethnicity and muscular mass influenced its urinary levels. Thus, SG adjustment was recommended for occupational biomonitoring (Truchon et al., 2014). However, this method requires the choice of a reference density (SG_{ref}) ranging from 1.016 to 1.024 (Heavner et al., 2006; Lee et al., 1996; Levine and Fahy, 1945). A

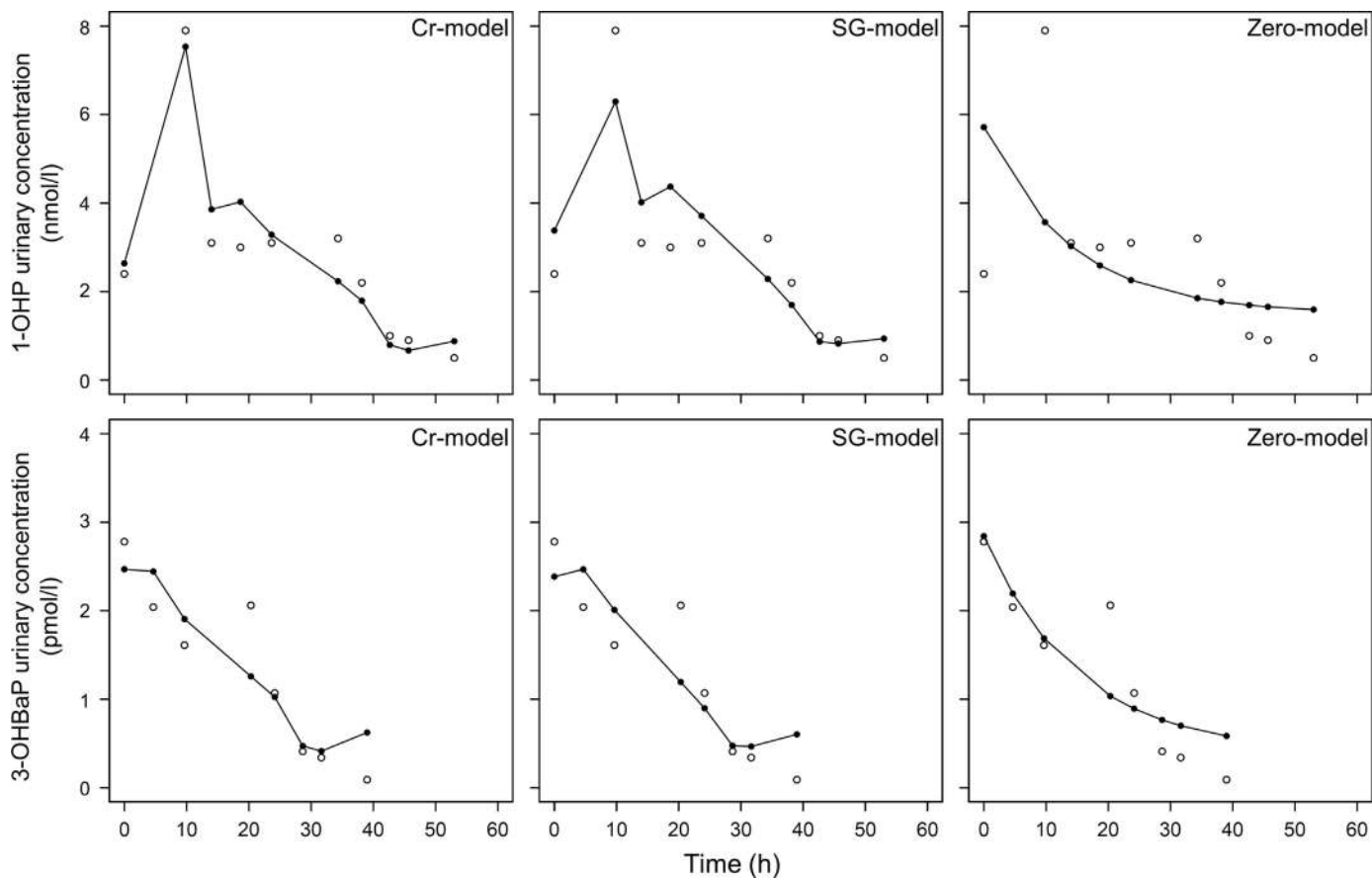


Fig. 3. Comparison of measured urinary concentrations for W1 (open circles) and model simulations (lines and closed circles).

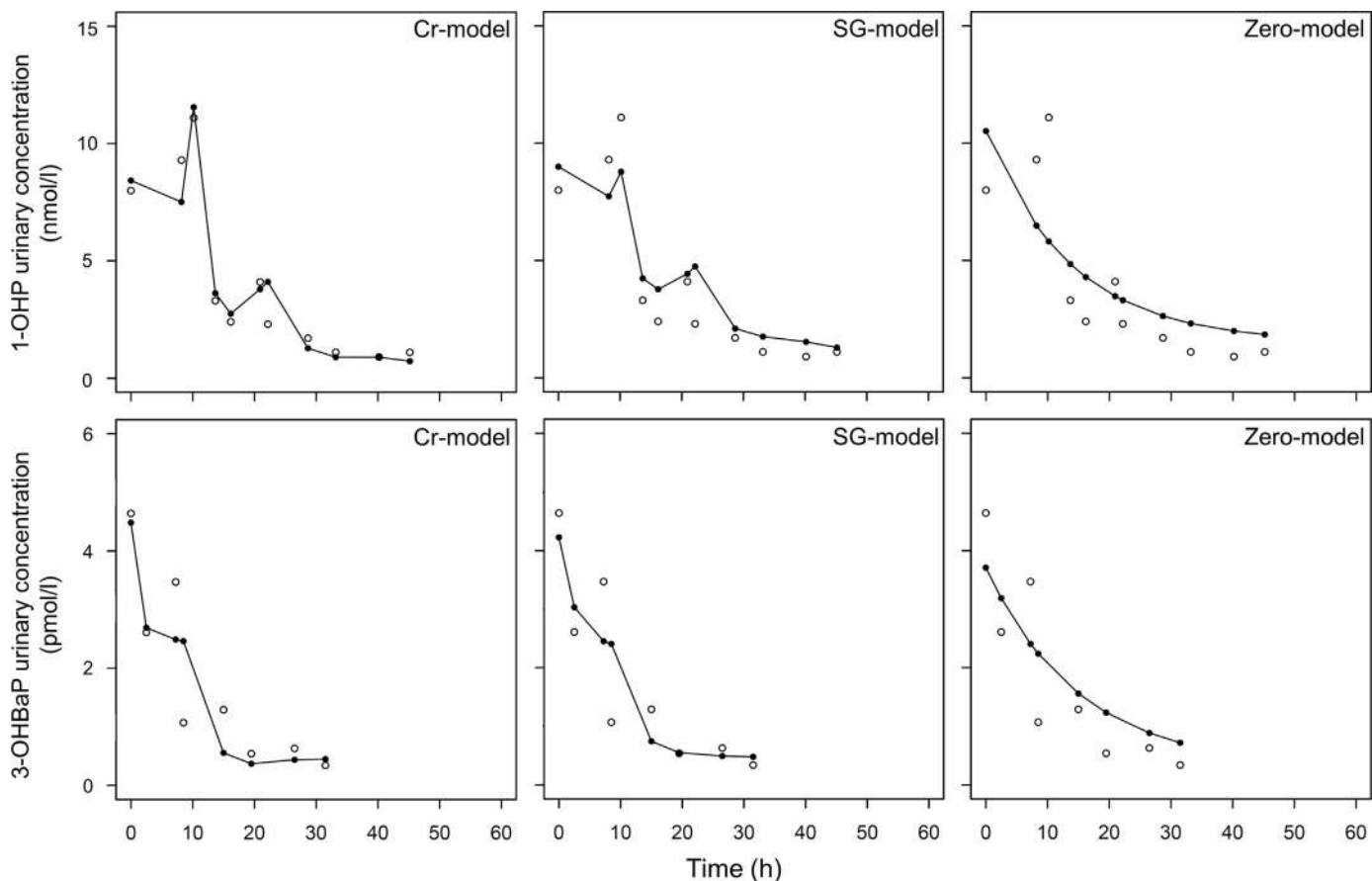


Fig. 4. Comparison of measured urinary concentrations for W2 (open circles) and model simulations (lines and closed circles).

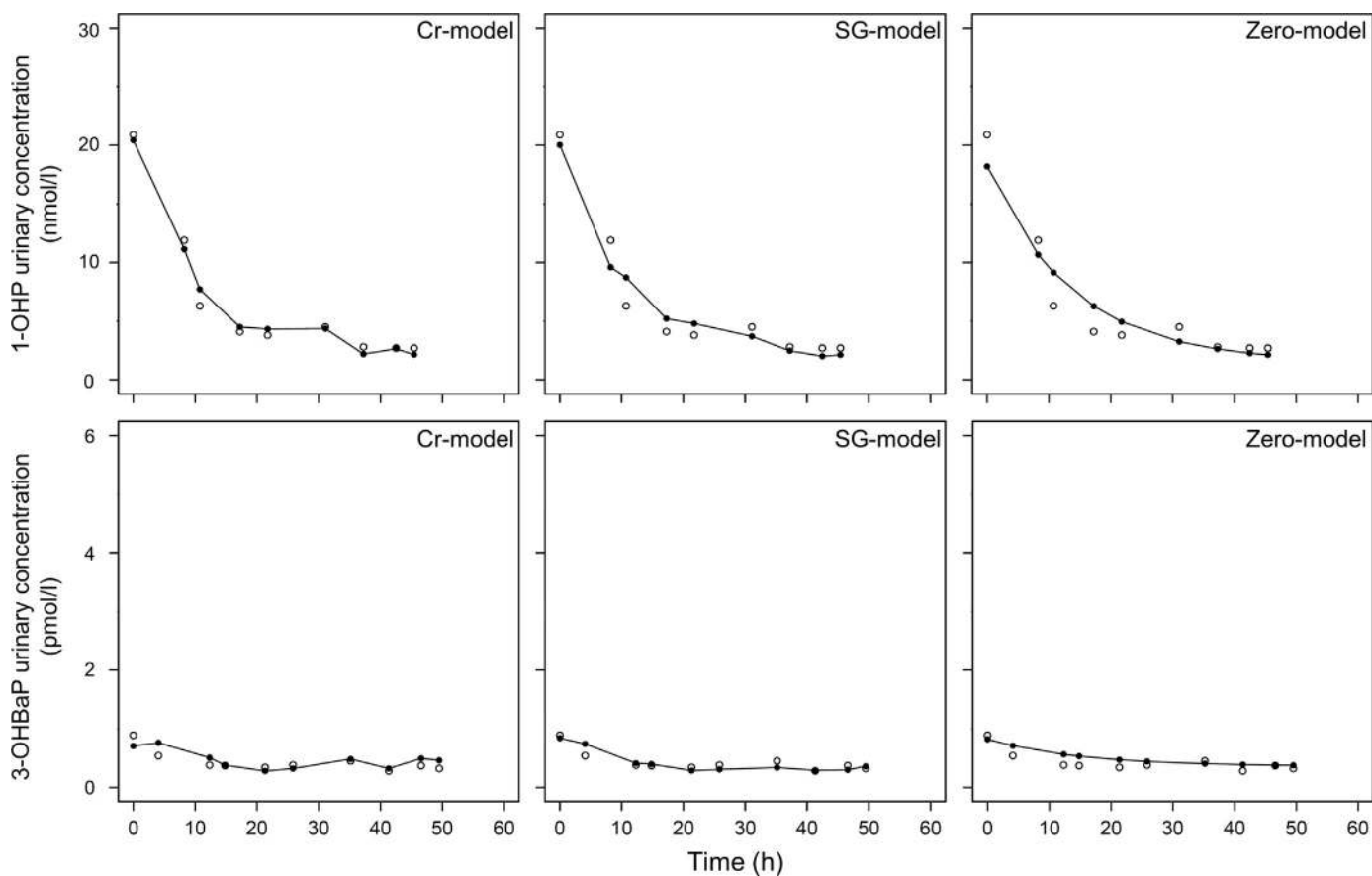


Fig. 5. Comparison of measured urinary concentrations for W3 (open circles) and model simulations (lines and closed circles).

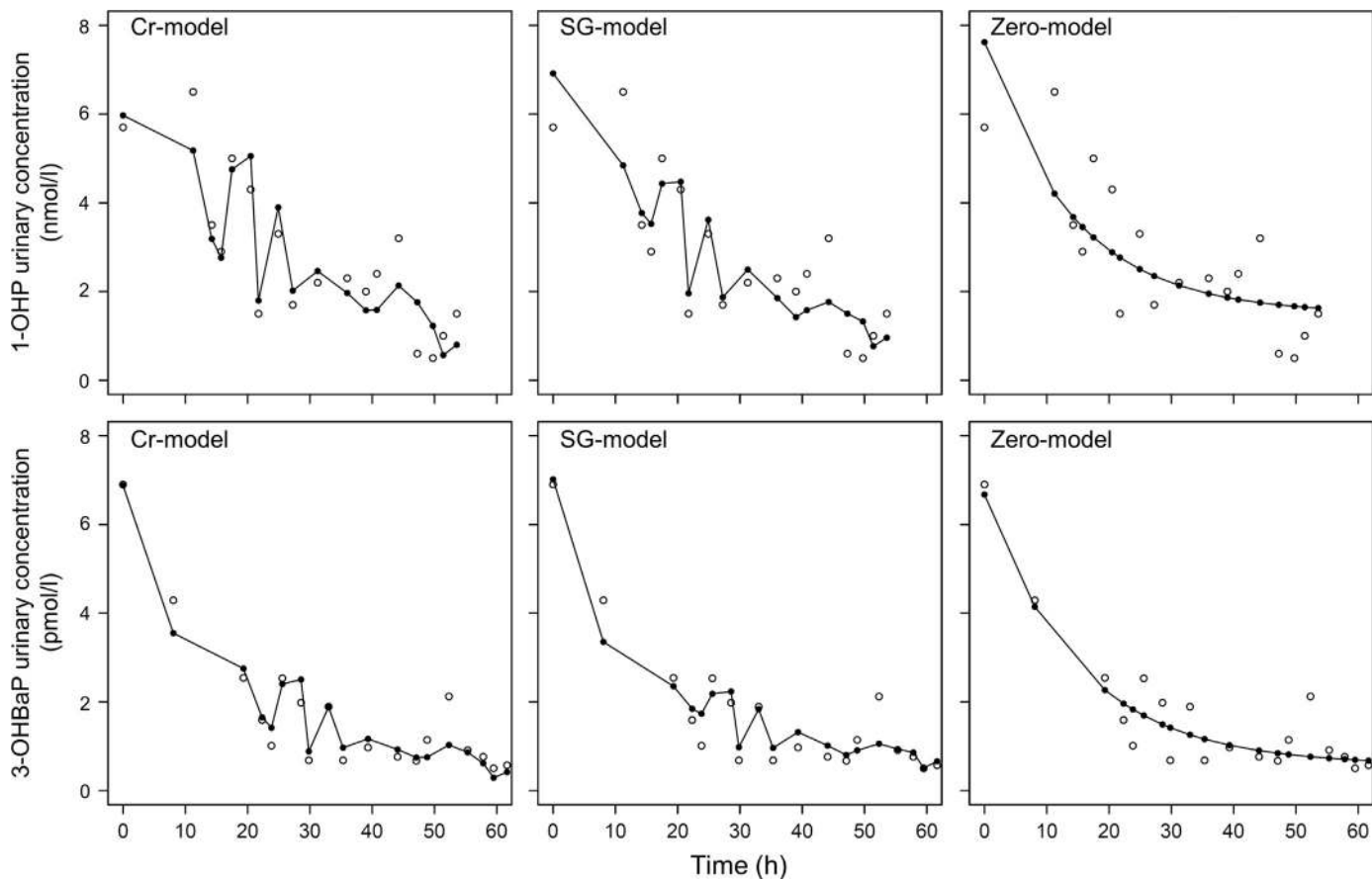


Fig. 6. Comparison of measured urinary concentrations for W4 (open circles) and model simulations (lines and closed circles).

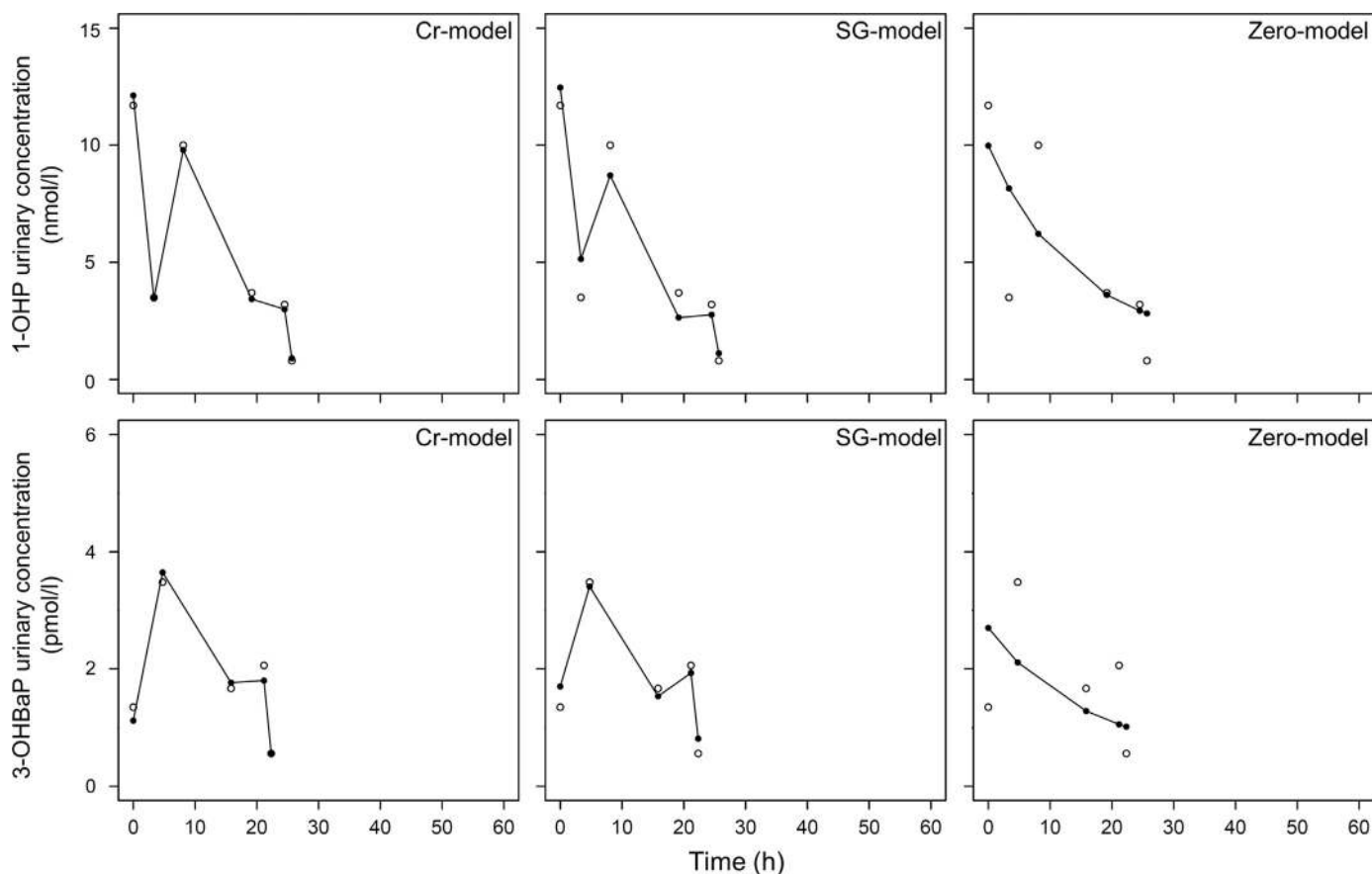


Fig. 7. Comparison of measured urinary concentrations for W5 (open circles) and model simulations (lines and closed circles).

SG_{ref} equal to 1.020 was widely used (Goldberger et al., 1995; Vij and Howell, 1998). For SG-model in the current work, the mean of urinary SG from samples of studied population (1.017) was used as SG_{ref} , as recommended (Truchon et al., 2014). Regarding 1-OHP, lower %RMSE was obtained for Cr-model compared to SG-model. Viau et al. (2004) had already shown that creatinine adjustment was an adapted diuresis correction method for this metabolite. However, for 3-OHBP, the difference was less evident, with quite similar %RMSE between the two correction modes. To our knowledge, diuresis normalization method was not previously studied for the latter exposure biomarker. Comparison of different diuresis correction ways should provide information about the renal excretion mechanisms of urinary metabolites. First, substances excreted in urine by passive diffusion should not be normalized (Boeniger et al., 1993). Results of this current study were thus in accordance with renal elimination of 1-OHP and 3-OHBP by glomerular filtration or tubular secretion. Secondly, since Cr-model was better than SG-model for 1-OHP, this metabolite is probably excreted by the same renal mechanism than creatinine, *ie* glomerular filtration without tubular reabsorption (Boeniger et al., 1993). Finally, SG-model was not better for 3-OHBP, while normalization by SG should be more adapted if it was excreted by active tubular secretion (Truchon et al., 2014), as previously proposed to explain its accumulation in kidney (Marie et al., 2010). Recently, a potential reabsorption of mono-hydroxylated BaP metabolite (including 3-OHBP) was proposed to explain kidney accumulation (Moreau et al., 2015). Results of the current study were more in accordance with 3-OHBP tubular reabsorption than active tubular secretion. This hypothesis could also explain the higher %RMSE of kinetic models for 3-OHBP than 1-OHP since they were built from linear first-order elimination kinetic. However, further works were required to define accurately renal

mechanisms of 3-OHBP excretion. In the end, Cr-model seemed a bit more appropriate to reproduce urinary kinetic than SG-model for both metabolites in case of W3 and W4 and was distinctly better for the others. Thus, use of urinary creatinine adjustment was proposed as good compromise to take into account diuresis effect on urinary 1-OHP and 3-OHBP concentrations.

Calculated urinary 1-OHP half-life from Cr-model (14.4 h) was two times higher than mean half-life obtained after pyrene inhalation in smokers (St Helen et al., 2012) and corresponded to those observed after dermal uptake in volunteers (Viau and Vyskocil, 1995). In rats, cutaneous absorption of pyrene increased threefold the apparent urinary half-life of 1-OHP compared to intraperitoneal injection (Payan et al., 2008). This skin reservoir effect combined with occupational exposure conditions could explain the high variability of 1-OHP half-lives previously observed (from 2.7 to 14.9 h) in six different workplaces with different personal protection equipment (Lafontaine et al., 2004). In the current study, the low inter-individual variability of 1-OHP half-lives (CI95%=12.0–18.2 h) indicated that PAHs exposure was mainly via cutaneous absorption for every worker. On the other hand, calculated urinary 3-OHBP half-life from Cr-model (8.9 h) was the same that the previously obtained from occupational exposed workers (Bouchard and Viau, 1996; Lafontaine et al., 2004; Payan et al., 2009) and from rats exposed to BaP via intravenous injection (8.1 h) or via cutaneous application (8.9 h) (Bouchard and Viau, 1996; Lafontaine et al., 2004; Payan et al., 2009). Skin reservoir effect in case of dermal uptake of BaP previously showed (Bouchard and Viau, 1997; Chu et al., 1996) was probably hidden by the accumulation of this metabolite into the kidney. Unlike to 1-OHP, inter-individual variability of 3-OHBP half-lives from Cr-model was very high. This result was partly due to the short sampling period of W5 (22 h) responsible for consideration of a

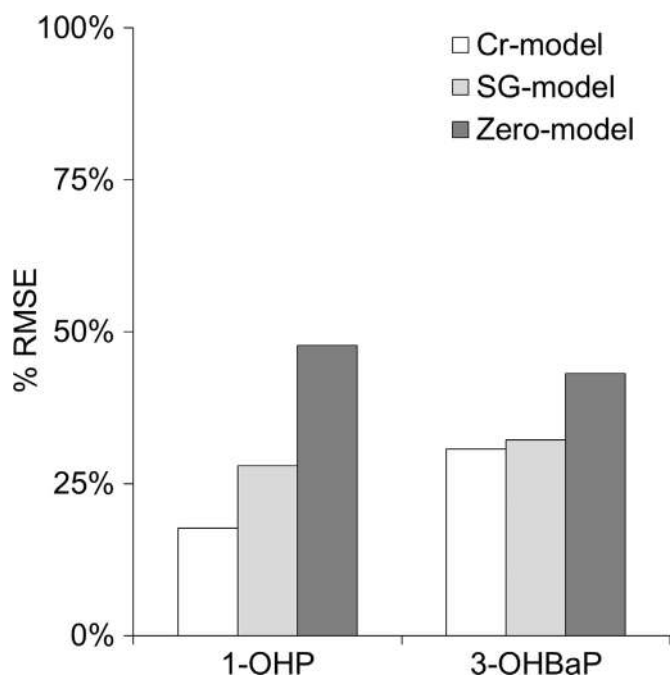


Fig. 8. Percentage of root mean square error (%RMSE) of Cr-model, SG-model and Zero-model for urinary 1-OHP and 3-OHBP.

high urinary 3-OHBP background (C_0) for this worker. Indeed, delay in the excretion of 3-OHBP compared to 1-OHP was demonstrated and C_0 for this metabolite was significant in the Cr-model (mean 3-OHBP half-life was increased by 42% when C_0 was excluded). Consequently, after exclusion of W_5 , upper limit of CI95% for calculated 3-OHBP half-life was drastically decreased, from 49.5 h to 20.9 h while mean was less affected (from 8.9 h to 6.8 h). This result were in agreement with previous studies showing that including background allowed to prevent miscalculation of half-lives (Bartell, 2012; Lafontaine et al., 2000; Li et al., 2012). However, in this study, urinary 1-OHP background coming from lifestyle seemed too low compared to pyrene occupational exposure to impact calculated half-life.

5. Conclusions

Knowledge about urinary elimination kinetic is required to find a compromise between excretion maximum of biomarkers and convenience to perform biomonitoring. The delay observed for maximum urinary excretion rates of 3-OHBP confirmed that sampling time should be performed the next morning after exposure. Considering workers as a random effect, toxicokinetic mixed effect models proposed in this work allowed to observe inter- and intra-individual variability of urinary biomarkers concentrations. Impact of diuresis, as part of intra-individual variability, was studied and urinary creatinine was found as a better way than SG to normalize urinary concentrations of 1-OHP and as a good compromise for 3-OHBP. Study of diuresis correction modes also allowed to strengthen the hypothesis of tubular reabsorption of 3-OHBP as a specific excretion mechanism. Mean apparent urinary half-life of 3-OHBP and 1-OHP was equal to 8.9 h and 14.4 h, respectively. Finally, due to homogeneous influence of dermal uptake among workers on the kinetic of 1-OHP, inter-individual variability of its half-life was low. On the contrary, high inter-individual variability of 3-OHBP half-life came from heterogeneous urinary background levels among workers. Such toxicokinetic mixed effects model could be used to other urinary

Table 2
Calculated parameters of Cr-model and elimination half-lives for urinary 1-OHP and 3-OHBP.

Metabolite	μC_m^a (SE) [CI95%]	μC_0^a (SE) [CI95%]	μk^b (SE) [CI95%]	$t_{1/2}^c$ [CI95%]
1-OHP	820** (60) [710–930]	NS	0.048** (0.005) [0.038–0.058]	14.4 [12.0–18.2]
3-OHBP	0.31* (0.12) [0.08–0.55]	0.02* (0.01) [0.01–0.04]	0.078* (0.033) [0.014–0.143]	8.9 [4.8–49.5]

^a nmol/mol creatinine

^b h⁻¹

^c h

* $p < 0.05$

** $p < 0.01$

biomarkers of chemicals or metals exposure in order to perform biomonitoring.

Funding sources and ethical approvals

This work was supported by the “Agence Nationale de Sécurité Sanitaire de l’Alimentation, de l’Environnement et du Travail (ANSES)”. ANSES’s missions include risk assessment in the fields of food, environment and workplace, for the purpose of assisting the authorities on health and safety issues. ANSES is a public authority reporting to the French Ministries of Health, Agriculture, Environment, labor and Consumer Affairs.

Study ethics approval was obtained by CECIC Rhône-Alpes-Auvergne, Clermont-Ferrand, IRB 5891.

Acknowledgments

The authors acknowledge financial support from the “Agence Nationale de Sécurité Sanitaire de l’Alimentation, de l’Environnement et du Travail (ANSES) (2011-CRD-13)” and thank “Région Rhône-Alpes, France (RA0000R604)” for a grant for the thesis of S. Lutier. We thank volunteers who collected numerous biological samples, and Sylvette Liaudy, information specialist, for her work.

References

- ACGIH, 2015. Documentation of the TLVs and BEIs. American Conference of Governmental Industrial Hygienists, Cincinnati, OH., 272 p. ISBN:978-1-607260-77-6.
- Barbeau, D., Lutier, S., Bonnetterre, V., Persoons, R., Marques, M., Herve, C., Maitre, A., 2015. Occupational exposure to polycyclic aromatic hydrocarbons: relations between atmospheric mixtures, urinary metabolites and sampling times. *Int Arch Occup Environ Health*. Epub ahead of print Mar 6. <http://dx.doi.org/10.1007/s00420-015-1042-1>.
- Barbeau, D., Maitre, A., Marques, M., 2011. Highly sensitive routine method for urinary 3-hydroxybenzo[a]pyrene quantitation using liquid chromatography-fluorescence detection and automated off-line solid phase extraction. *Analyst* 136, 1183–1191. <http://dx.doi.org/10.1039/C0AN00428F>.
- Barbeau, D., Persoons, R., Marques, M., Herve, C., Laffitte-Rigaud, G., Maitre, A., 2014. Relevance of urinary 3-hydroxybenzo(a)pyrene and 1-hydroxypyrene to assess

- exposure to carcinogenic polycyclic aromatic Hydrocarbon mixtures in metallurgy workers. *Ann. Occup. Hyg.* 58, 579–590. <http://dx.doi.org/10.1093/annhyg/meu004>.
- Bartell, S.M., 2012. Bias in half-life estimates using log concentration regression in the presence of background exposures, and potential solutions. *J. Expo. Sci. Environ. Epidemiol.* 22, 299–303. <http://dx.doi.org/10.1038/jes.2012.2>.
- Boeniger, M.F., Lowry, L.K., Rosenberg, J., 1993. Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. *Am. Ind. Hyg. Assoc. J.* 54, 615–627. <http://dx.doi.org/10.1080/15298669391355134>.
- Boogaard, P.J., 2008. Urinary biomarkers in the risk assessment of PAHs. *Occup. Environ. Med.* 65, 221–222. <http://dx.doi.org/10.1136/oem.2007.034157>.
- Bouchard, M., Viau, C., 1996. Urinary excretion kinetics of pyrene and benzo(a)pyrene metabolites following intravenous administration of the parent compounds or the metabolites. *Toxicol. Appl. Pharmacol.* 139, 301–309. <http://dx.doi.org/10.1006/taap.1996.0169>.
- Bouchard, M., Viau, C., 1997. Urinary excretion of benzo(a)pyrene metabolites following intravenous, oral, and cutaneous benzo(a)pyrene administration. *Can. J. Physiol. Pharm.* 75, 185–192. <http://dx.doi.org/10.1139/y97-017>.
- Bouchard, M., Viau, C., 1999. Urinary 1-hydroxypyrene as a biomarker of exposure to polycyclic aromatic hydrocarbons: biological monitoring strategies and methodology for determining biological exposure indices for various work environments. *Biomarkers* 4, 159–187. <http://dx.doi.org/10.1080/135475099230859>.
- Buchet, J.P., Gennart, J.P., Mercado-Calderon, F., Delavignette, J.P., Cupers, L., Lauwerys, R., 1992. Evaluation of exposure to polycyclic aromatic hydrocarbons in a coke production and a graphite electrode manufacturing plant: assessment of urinary excretion of 1-hydroxypyrene as a biological indicator of exposure. *Br. J. Ind. Med.* 49, 761–768.
- Buckley, T.J., Lioy, P.J., 1992. An examination of the time course from human dietary exposure to polycyclic aromatic hydrocarbons to urinary elimination of 1-hydroxypyrene. *Br. J. Ind. Med.* 49, 113–124. <http://dx.doi.org/10.1136/oem.49.2.113>.
- Campins-Falco, P., Herraez-Hernandez, R., Sevillano-Cabeza, A., 1993. Column-switching techniques for High-performance liquid chromatography of drugs in biological samples. *J. Chromatogr.* 619, 177–190.
- Chien, Y.C., Yeh, C.T., 2010. Amounts and proportion of administered pyrene dose excreted as urinary 1-hydroxypyrene after dietary exposure to polycyclic aromatic hydrocarbons. *Arch. Toxicol.* 84, 767–776. <http://dx.doi.org/10.1007/s00204-010-0570-4>.
- Chu, I., Dick, D., Bronaugh, R., Tryphonas, L., 1996. Skin reservoir formation and bioavailability of dermally administered chemicals in hairless guinea pigs. *Food Chem. Toxicol.* 34, 267–276. [http://dx.doi.org/10.1016/0278-6915\(95\)00112-3](http://dx.doi.org/10.1016/0278-6915(95)00112-3).
- Elovaara, E., Heikkilä, P., Pyy, L., Mutanen, P., Riihimäki, V., 1995. Significance of dermal and respiratory uptake in creosote workers: exposure to polycyclic aromatic hydrocarbons and urinary excretion of 1-hydroxypyrene. *Occup. Environ. Med.* 52, 196–203. <http://dx.doi.org/10.1136/oem.52.3.196>.
- Forster, K., Preuss, R., Rossbach, B., Bruning, T., Angerer, J., Simon, P., 2008. 3-Hydroxybenzo(a)pyrene in the urine of workers with occupational exposure to polycyclic aromatic hydrocarbons in different industries. *Occup. Environ. Med.* 65, 224–229. <http://dx.doi.org/10.1136/oem.2006.030809>.
- Gelboin, H.V., 1980. Benzo[alpha]pyrene metabolism, activation and carcinogenesis: role and regulation of mixed-function oxidases and related enzymes. *Physiol. Rev.* 60, 1107–1166.
- Gendre, C., Lafontaine, M., Delsaut, P., Simon, P., 2004. Exposure to polycyclic aromatic hydrocarbons and excretion of urinary 3-hydroxybenzo(a)pyrene: assessment of an appropriate sampling time. *Polycycl. Aroma. Compd.* 24, 433–439. <http://dx.doi.org/10.1080/10406630490468766>.
- Gendre, C., Lafontaine, M., Morele, Y., Payan, J.P., Simon, P., 2002. Relationship between urinary levels of 1-hydroxypyrene and 3-hydroxybenzo(a)pyrene for workers exposed to polycyclic aromatic Hydrocarbons. *Polycycl. Aroma. Compd.* 22, 761–769. <http://dx.doi.org/10.1080/10406630213612>.
- Goldberger, B.A., Loewenthal, B., Darwin, W.D., Cone, E.J., 1995. Intrasubject variation of creatinine and specific gravity measurements in consecutive urine specimens of heroin users. *Clin. Chem.* 41, 116–117.
- Guignon, N., Sandret, N., 2005. SUMER 2003 : les expositions aux produits cancérogènes, mutagènes et reprotoxiques, Documents pour le médecin du Travail. 104, pp. 471–483.
- Hansen, A.M., Mathiesen, L., Pedersen, M., Knudsen, L.E., 2008. Urinary 1-hydroxypyrene (1-HP) in environmental and occupational studies – a review. *Int. J. Hyg. Environ. Health* 211, 471–503. <http://dx.doi.org/10.1016/j.ijheh.2007.09.012>.
- Heavner, D.L., Morgan, W.T., Sears, S.B., Richardson, J.D., Byrd, G.D., Ogden, M.W., 2006. Effect of creatinine and specific gravity normalization techniques on xenobiotic biomarkers in smokers' spot and 24-h urines. *J. Pharm. Biomed. Anal.* 40, 928–942. <http://dx.doi.org/10.1016/j.jpba.2005.08.008>.
- Heredia Ortiz, R., Maitre, A., Barbeau, D., Lafontaine, M., Bouchard, M., 2014. Use of physiologically-based pharmacokinetic modeling to simulate the profiles of 3-hydroxybenzo(a)pyrene in workers exposed to polycyclic aromatic hydrocarbons. *PLoS One* 9, e102570. <http://dx.doi.org/10.1371/journal.pone.0102570>.
- IARC 2004. Tobacco Smoke and Involuntary Smoking. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Lyon volume 83. Lyon, IARC, p. 1470. ISBN 978-92-832-1283-6.
- IARC, 2010. Some Non-Heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Lyon volume 92, Lyon, IARC, p. 868. ISBN : 978-92-832-1292-8.
- Jongeneelen, F.J., Anzion, R.B.M., Leijdekkers, C.M., Bos, R.P., Henderson, P.T., 1985. 1-hydroxypyrene in human urine after exposure to coal tar and a coal tar derived product. *Int. Arch. Occup. Environ. Health* 57, 47–55. <http://dx.doi.org/10.1007/BF00383545>.
- Jongeneelen, F.J., Anzion, R.B.M., Scheepers, P.T.J., Bos, R.P., Henderson, P.T., Nijenhuis, E.H., Veenstra, S.J., Brouns, R.M.E., Winkes, A., 1988. 1-Hydroxypyrene in urine as a biological indicator of exposure to polycyclic aromatic hydrocarbons in several work environments. *Ann. Occup. Hyg.* 32, 35–43. <http://dx.doi.org/10.1093/annhyg/32.1.35>.
- Lafontaine, M., Champmartin, C., Simon, P., Delsaut, P., Funck-Brentano, C., 2006. 3-Hydroxybenzo(a)pyrene in the urine of smokers and non-smokers. *Toxicol. Lett.* 162, 181–185. <http://dx.doi.org/10.1016/j.toxlet.2005.09.019>.
- Lafontaine, M., Gendre, C., Delsaut, P., Simon, P., 2004. Urinary 3-hydroxybenzo(a)pyrene as a biomarker of exposure to polycyclic aromatic hydrocarbons: an approach for determining a biological limit value. *Polycycl. Aroma. Compd.* 24, 441–450. <http://dx.doi.org/10.1080/10406630490471447>.
- Lafontaine, M., Gendre, C., Morele, Y., Laffitte-Rigaud, G., 2002. Excretion of urinary 1-hydroxypyrene in relation to the penetration routes of polycyclic aromatic hydrocarbons. *Polycycl. Aroma. Compd.* 22, 579–588. <http://dx.doi.org/10.1080/10406630213600>.
- Lafontaine, M., Payan, J.P., Delsaut, P., Morele, Y., 2000. Polycyclic aromatic hydrocarbon exposure in an artificial shooting target factory: assessment of 1-hydroxypyrene urinary excretion as a biological indicator of exposure. *Ann. Occup. Hyg.* 44, 89–100. <http://dx.doi.org/10.1093/annhyg/44.2.89>.
- Lee, E., Park, H.K., Kim, H.J., 1996. Adjustment of urinary mercury in health risk assessment of mercury. *J. Korean Med. Sci.* 11, 319–325. <http://dx.doi.org/10.3346/jkms.1996.11.4.319>.
- Levine, L., Fahy, J.P., 1945. Evaluation of urinary lead determinations. *J. Ind. Hyg. Toxicol.* 27, 217–223.
- Li, Z., Romanoff, L., Bartell, S., Pittman, E.N., Trinidad, D.A., McClean, M., Webster, T. F., Sjödin, A., 2012. Excretion profiles and half-lives of ten urinary polycyclic aromatic hydrocarbon metabolites after dietary exposure. *Chem. Res. Toxicol.* 25, 1452–1461. <http://dx.doi.org/10.1021/tx300108e>.
- Marczynski, B., Raulf-Heimsoth, M., Spickenheuer, A., Pesch, B., Kendzia, B., Mensing, T., Engelhardt, B., Lee, E.H., Schindler, B.K., Heinze, E., Welge, P., Bramer, R., Angerer, J., Breuer, D., Kafferlein, H.U., Bruning, T., 2011. DNA adducts and strand breaks in workers exposed to vapours and aerosols of bitumen: associations between exposure and effect. *Arch. Toxicol.* 85 (Suppl. 1), S53–64. <http://dx.doi.org/10.1007/s00204-011-0682-5>.
- Marie, C., Bouchard, M., Heredia-Ortiz, R., Viau, C., Maitre, A., 2010. A toxicokinetic study to elucidate 3-hydroxybenzo(a)pyrene atypical urinary excretion profile following intravenous injection of benzo(a)pyrene in rats. *J. Appl. Toxicol.* 30, 402–410. <http://dx.doi.org/10.1002/jat.1511>.
- Moreau, M., Ayyotte, P., Bouchard, M., 2015. Kinetics of diol and hydroxybenzo(a)pyrene metabolites in relation to DNA adduct formation and gene expression in rats. *J. Toxicol. Environ. Health A* 78, 725–746. <http://dx.doi.org/10.1080/15287394.2015.1028119>.
- Motorykin, O., Santiago-Delgado, L., Rohlman, D., Schrlau, J.E., Harper, B., Harris, S., Harding, A., Kile, M.L., Massey Simonich, S.L., 2015. Metabolism and excretion rates of parent and hydroxy-PAHs in urine collected after consumption of traditionally smoked salmon for Native American volunteers. *Sci. Total Environ.* 514, 170–177. <http://dx.doi.org/10.1016/j.scitotenv.2015.01.083>.
- Payan, J.P., Lafontaine, M., Simon, P., Marquet, F., Champmartin-Gendre, C., Beydon, D., Ferrari, E., 2008. In vivo and in vitro percutaneous absorption of [¹⁴C]pyrene in Sprague Dawley male rats: skin reservoir effect and consequence on urinary 1-OH pyrene excretion. *Arch. Toxicol.* 82, 739–747. <http://dx.doi.org/10.1007/s00204-008-0300-3>.
- Payan, J.P., Lafontaine, M., Simon, P., Marquet, F., Champmartin-Gendre, C., Beydon, D., Wathier, L., Ferrari, E., 2009. 3-Hydroxybenzo(a)pyrene as a biomarker of dermal exposure to benzo(a)pyrene. *Arch. Toxicol.* 83, 873–883. <http://dx.doi.org/10.1007/s00204-009-0440-0>.
- Petry, T., Schmid, P., Schlatter, C., 1996. Airborne exposure to polycyclic aromatic hydrocarbons (PAHs) and urinary excretion of 1-hydroxypyrene of carbon anode plant workers. *Ann. Occup. Hyg.* 40, 345–357. <http://dx.doi.org/10.1093/annhyg/40.3.345>.
- Pinheiro, J.C., Bates, D.M., 2000. *Mixed-Effects Models in S and S-PLUS*. Springer-Verlag, New York, p. 537, ISBN: 978-0-387-98957-0.
- Sauvé, J.F., Lévesque, M., Huard, M., Drolet, D., Lavoué, J., Tardif, R., Truchon, G., 2015. Creatinine and specific gravity normalization in biological monitoring of occupational exposures. *J. Occup. Environ. Hyg.* 12, 123–129. <http://dx.doi.org/10.1080/15459624.2014.955179>.
- Siwińska, E., Mielżyńska, D., Kapka, L., 2004. Association between urinary 1-hydroxypyrene and genotoxic effects in Coke oven workers. *Occup. Environ. Med.* 61, e10. <http://dx.doi.org/10.1136/oem.2002.006643>.
- Sobus, J.R., McClean, M.D., Herrick, R.F., Waidyanatha, S., Onyemauwa, F., Kupper, L. L., Rappaport, S.M., 2009. Investigation of PAH biomarkers in the urine of workers exposed to hot asphalt. *Ann. Occup. Hyg.* 53, 551–560. <http://dx.doi.org/10.1016/j.toxlet.2010.09.006>.
- St Helen, G., Goniewicz, M.L., Dempsey, D., Wilson, M., Jacob 3rd, P., Benowitz, N.L., 2012. Exposure and kinetics of polycyclic aromatic hydrocarbons (PAHs) in cigarette smokers. *Chem. Res. Toxicol.* 25, 952–964. <http://dx.doi.org/10.1021/tx300043k>.
- Strickland, P., Kang, D., Sithisarankul, P., 1996. Polycyclic aromatic hydrocarbon metabolites in urine as biomarkers of exposure and effect. *Env. Health Perspect.* 104 (Suppl. 5), 927–932.
- Talaska, G., Thoroman, J., Schuman, B., Kafferlein, H.U., 2014. Biomarkers of

- polycyclic aromatic hydrocarbon exposure in European coke oven workers. *Toxicol. Lett.* 231, 213–216. <http://dx.doi.org/10.1016/j.toxlet.2014.10.025>.
- Truchon, G., Huard, M., Lévesque, M., Sauvé, J.F., Larivière, P., Tardif, R., 2014. Surveillance biologique de l'exposition professionnelle – quel mode de correction urinaire choisir lors de prélèvements ponctuels ? Institut de recherche Robert-Sauvé en santé et en sécurité du travail (IRSST), Montréal, p. 55. ISBN 978-2-89631-728-8.
- Unwin, J., Cocker, J., Scobbie, E., Chambers, H., 2006. An assessment of occupational exposure to polycyclic aromatic hydrocarbons in the UK. *Ann. Occup. Hyg.* 50, 395–403. <http://dx.doi.org/10.1093/annhyg/mel010>.
- VanRooij, J.G., Bodelier-Bade, M.M., Jongeneelen, F.J., 1993. Estimation of individual dermal and respiratory uptake of polycyclic aromatic hydrocarbons in 12 coke oven workers. *Br. J. Ind. Med.* 50, 623–632. <http://dx.doi.org/10.1136/oem.50.7.623>.
- Viau, C., Lafontaine, M., Payan, J.P., 2004. Creatinine normalization in biological monitoring revisited: the case of 1-hydroxypyrene. *Int. Arch. Occup. Environ. Health* 77, 177–185. <http://dx.doi.org/10.1007/s00420-003-0495-9>.
- Viau, C., Vyskocil, A., 1995. Patterns of 1-hydroxypyrene excretion in volunteers exposed to pyrene by the dermal route. *Sci. Total Environ.* 163, 187–190. [http://dx.doi.org/10.1016/0048-9697\(95\)04495-M](http://dx.doi.org/10.1016/0048-9697(95)04495-M).
- Vij, H.S., Howell, S., 1998. Improving the specific gravity adjustment method for assessing urinary concentrations of toxic substances. *Am. Ind. Hyg. Assoc. J.* 59, 375–380. <http://dx.doi.org/10.1080/15428119891010622>.
- Weaver, V.M., Kotchmar, D.J., Fadowski, J.J., Silbergeld, E.K., 2015. Challenges for environmental epidemiology research: are biomarker concentrations altered by kidney function or urine concentration adjustment? *J. Expo. Sci. Environ. Epidemiol.* <http://dx.doi.org/10.1038/jes.2015.8>