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Comparison of the urinary excretion time courses of pyrene-1,6-dione, pyrene-1,8-dione and 1-hydroxypyrene in rats intravenously exposed to pyrene

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Abstract

The urinary excretion time courses of pyrene-1,6-dione (P16D), pyrene-1,8-dione (P18D) and 1-hydroxypyrene (1-OHP) were compared in Sprague–Dawley and Wistar rats. Groups of five male rats, of about 200 g of body weight, were injected intravenously with 0.05, 0.5, 5 and 50 μmol pyrene kg^{-1} of body weight. Urine was collected at 2, 4, 6, 8, 10, 12, 18, 24, 30, 42 and 48 h post-dosing. Pyrene metabolites were measured by high-performance liquid chromatography (HPLC)/fluorescence after enzymatic hydrolysis of the glucurono- and sulfo-conjugates, extraction on Sep-Pak C18 cartridges and, for the analysis of dione metabolites, derivatization to stable diacetoxypyrene molecules. Over the 48-h sampling period, on average 17.4–25.6% of the injected pyrene was excreted overall as P16D, 6.4–8.8% as P18D and 0.6–0.8% as 1-OHP in the urine of Sprague–Dawley rats. By comparison, on average 10.3–14.7% of the intravenous pyrene dose was recovered as P16D, 4.8–6.4% as P18D and 0.3–0.4% as 1-OHP in the urine of Wistar rats. In both strains of rats there was no clear effect of the dose on the 0–48-h cumulative urinary excretion of P18D and 1-OHP over the entire dose range, while the percentage of dose recovered overall as P16D in urine at the highest dose (50 μmol kg^{-1}) was statistically lower than at the other doses. The 0–48-h cumulative percentage of pyrene dose excreted as metabolites in the urine of Sprague–Dawley rats was also significantly higher than in Wistar rats ($p < 0.01$) exposed under identical conditions. As for the urinary excretion-time courses of the different metabolites, for a given dose and strain of rats, excretion curves of P16D, P18D and 1-OHP generally evolved in parallel. There was also no clear effect of the dose on the excretion rate, thus half-life, of pyrene metabolites, except for P16D in Sprague–Dawley rats at the highest dose where elimination tended to be slower compared with the other doses ($p < 0.01$). The average first-order elimination half-life of P16D, P18D and 1-OHP was 4.0, 5.7 and 4.1 h, respectively, in Sprague–Dawley rats, and 5.1, 6.1 and 5.1 h, respectively, in Wistar rats (all doses combined but excluding the highest dose for P16D). This study showed the relative importance of metabolic pathways leading to diones

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compared with 1-OHP. These dioxygenated metabolites appear to be interesting biomarkers of pyrene exposure at environmentally and occupationally relevant doses. Their adequacy as biomarkers of human exposure has yet to be confirmed.

Keywords: *Pyrene-1,6-dione, pyrene-1,8-dione, 1-hydroxypyrene, urinary excretion time courses, polycyclic aromatic hydrocarbons, pyrene*

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Introduction

Some 30–50 years ago, 1-hydroxypyrene (1-OHP) was identified as a major hydroxylated metabolite of pyrene (Boyland & Sims 1964, Jacob et al. 1982, Keiming et al. 1983). Jongeneelen et al. (1987) proposed 1-OHP in the urine as a bioindicator of the overall exposure to polycyclic aromatic hydrocarbons (PAH). This was based mainly on the observations that pyrene and total PAH airborne concentrations were well-correlated and that there was a linear relationship between pyrene dose and 1-OHP urinary excretion (Tolos et al. 1990, Zhao et al. 1990).

In most PAH mixtures, pyrene is found in important proportions (1–10%) (Buchet et al. 1992, Roussel et al. 1992, Petry et al. 1994), but 1-OHP makes up only for a small percentage of the applied/administered pyrene dose in rat urine (0.01–2.7% depending on the route of exposure) (Jacob et al. 1989, Bouchard & Viau 1998, Elovaara et al. 2003) and in human urine (0.15–4.5% after dermal application, ingestion or inhalation) (Kang et al. 1995, Viau et al. 1995). For this reason, several studies have indicated that 1-OHP in urine lacks sensitivity as an indicator of low-dose environmental exposure (Jacob et al. 1989, Hansen et al. 1995, Chénier & Viau 1997, Hara et al. 1997).

A few years ago in a mass balance study, Bouchard et al. (1998a) indicated that 24-h urinary extracts from rats dosed with ^{14}C-pyrene contained only a small proportion of 1-OHP and that the bulk of the radioactivity corresponded to more polar metabolites. These metabolites were however not identified specifically. Gerde et al. (1998) also showed that dominant organic-extractable metabolites in tissues and urine of dogs instilled with ^{3}H-pyrene co-eluted with pyrene-1,6- and 1,8-dione (P16D and P18D) standards. The latter metabolites were however not well separated and urinary time course data were not gathered. Recently, a good high-performance liquid chromatography (HPLC) separation and quantification of these two diones was achieved (Ruzgyte et al. 2005), which now allows to assess their usefulness as bioindicators of exposure to pyrene.

In this context, the objective of the present study was to compare the urinary excretion time courses of P16D, P18D and 1-OHP following intravenous injection of pyrene at four dose levels, in both Sprague–Dawley and Wistar rats.

Materials and methods

Chemicals

Pyrene (purity >99%) was purchased from Aldrich (Milwaukee, WI, USA). 1-OHP (purity >99%) was obtained from the NCI Chemical Carcinogen Reference Standards distributed by the Midwest Research Institute (Kansas City, MO, USA). The P16D and P18D standards (purity >98%) were kindly donated by Dr Peter P. Fu

of the National Center for Toxicological Research (Jefferson, AR, USA). The non-ionic surfactant Alkamuls EL-620 (formerly emulphor EL-620) used to solubilize pyrene in aqueous solutions was donated by Rhone-Poulenc (St-Hyacinthe, QC, Canada). β-Glucuronidase/arylsulfatase (100 000 Fishman U ml^{-1} and 800 000 Roy U ml^{-1} from *Helix pomatia*) was purchased from Boehringer Mannheim (Laval, QC, Canada). Trimethylsilyl trifluoromethanesulfonate (TMSOTf) (purity 99%) and ethyl alcohol (purity 95%) were obtained from Sigma-Aldrich (Oakville, ON, Canada). Acetic anhydride (purity 99.7%) was purchased from Fisher (Nepean, ON, Canada). HPLC-grade methanol was purchased from Fisher (Fair Lawn, NJ, USA) and Triton X-100 was received from BDH, Inc. (ON, Canada). All buffers and HPLC mobile phases were prepared using MilliQ grade water (Millipore, Mississauga, ON, Canada).

Animals

Male Sprague–Dawley and Wistar rats (Charles River Canada, Inc., QC, Canada) of 195–225 g of body weight were used. Before intravenous injection, rats were kept in plastic cages in groups of two and following injection animals were put in individual metabolic cages. The principles and guidelines of the Canadian Council on Animal Care were followed.

Animal treatment

Four groups of five Sprague–Dawley and Wistar rats were administered intravenously with 0.05, 0.5, 5 or 50 μmol kg^{-1} of pyrene. Pyrene was dissolved in a 20% emulphor/80% isotonic glucose solution. The highest dose was the same as that used in an earlier work (Bouchard et al. 1998a); the 0.05 μmol kg^{-1} dose was chosen to verify the analytical limits. A total of 1 ml of solution was injected per kg of body weight.

During the 6-h period preceding injection up to 12-h post-dosing, rats were provided with water containing D-glucose (40 g l^{-1}) and saccharin (1.5 g l^{-1}), which induces a polydipsic behaviour and hence an aqueous diuresis allowing for frequent (2-h) urine collections (Chouinard & Viau 1992, Bouchard & Viau 1996). Over that period of time, food was removed. During the 12–48-h period post-dosing, after 18-h of fast, rats were again supplied with food.

Urine sampling

Control urine samples were collected during the 4-h period preceding injection. Over the 48-h period following injection, all urine voided was collected at frequent intervals ($t = 2$, 4, 6, 8, 10, 12, 18, 24, 30, 42 and 48 h) to establish the detailed urinary excretion time profiles of P16D, P18D and 1-OHP, as it was previously determined for 1-OHP (Bouchard et al. 1998b). To ensure complete collection of urine, the cage bottoms were rinsed with about 5 ml of distilled water at the end of each sampling interval and this fraction was added to the original urine. Urine samples were collected in 120 ml polyethylene bottles over thymol and frozen at $-20^{\circ}C$ until analysis.

Urine analysis

For the analysis of 1-OHP, samples were treated using a method adapted from Jongeneelen et al. (1987) and Bouchard et al. (1994), and for the analysis of P16D and P18D metabolites, the method developed by Ruzgyte et al. (2005) was used.

Briefly, urine samples were treated with β-glucuronidase/arylsulfatase, extracted on Sep-Pak C18 columns and, for the analysis of P16D and P18D, a derivatization step, with acetic anhydride in the presence of TMSOTf, was added to convert the dione metabolites to stable diacetoxypyrene molecules. Urine sample extracts were then analysed using a HPLC/fluorescence system composed of an automatic injector AS-100 (Bio-Rad, Richmond, Canada), a quaternary pump series 1100 from Hewlett Packard (Kirkland, Canada) and a LS-240 Perkin-Elmer fluorimeter (Buckingham, UK). The detector signal was recorded and treated with a Perkin-Elmer Nelson Turbochrom 4 Software. For the analysis of P16D and P18D, a Chrompack (15×0.46 cm, 5 μm) ChromSpher PAH-phase column (Varian, Mississauga, ON, Canada) was used and the injection volume was 20 μl. For the analysis of 1-OHP, a CSC-S (15×0.46 cm, 5 μm) ODS2-phase column (CSC, Inc., Canada) was used and the injection volume was 40 μl. For the quantification of P16D and P18D, fluorescence excitation and emission wavelengths were set at 345 and 400 nm, respectively, and for the quantification of 1-OHP, they were set at 242 and 388 nm, respectively. For P16D and P18D analysis, in order to avoid build up of urinary matrix impurities on the HPLC column, Triton X-100 (0.3 ml l^{-1}) was added to the methanol mobile phase. Figure 1 depicts representative chromatograms of a urine sample extract from a treated rat showing P16D, P18D and 1-OHP peaks.

The mean analytical limits of detection (LOD) for P16D, P18D and 1-OHP were 46 nmol l^{-1} (standard deviation (SD) = 22, $n = 9$), 86 nmol l^{-1} (SD = 32, $n = 9$) and 2.5 nmol l^{-1} (SD = 1.2, $n = 9$), respectively. Mean recovery rates of P16D, P18D and 1-OHP from spiked urine samples of Sprague–Dawley rats were 72% (SD = 16,

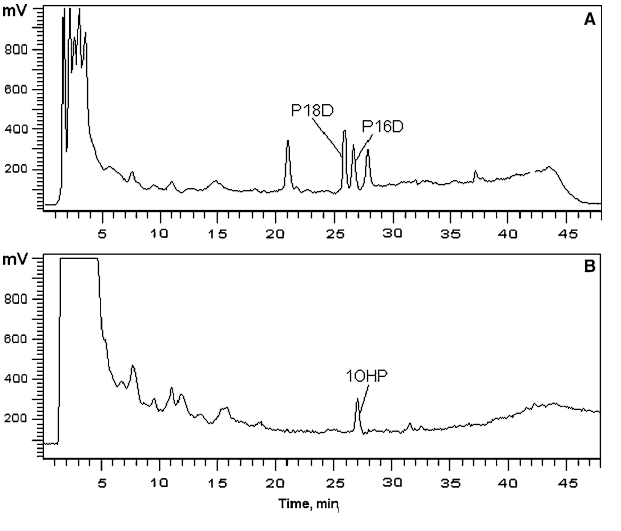

Figure 1. High-performance liquid chromatography (HPLC)/fluorescence chromatograms of a urinary extract from a rat intravenously injected with 0.5 μmol kg^{-1} of pyrene. (A) Pyrene-1,6-dione and pyrene-1,8-dione metabolites (diluted twice); (B) 1-OHP metabolite (non-diluted).

$n=10$), 79% (SD = 13, $n=8$), and 80% (SD = 18, $n=13$). For Wistar rat samples, the corresponding values were 90% (SD = 32, $n=6$), 92% (SD = 30, $n=5$), and 90% (SD = 23, $n=10$), respectively. Results presented herein were adjusted for recovery. The day-to-day coefficient of variation (reproducibility) over a period of 3 days was on average 13% for P16D, 7.3% for P18D and 12% for 1-OHP.

Toxicokinetic calculations

The excretion rates of P16D, P18D and 1-OHP were calculated by least-square fits of the following equation to the cumulative urinary excretion time course data for each rat:

$$\text{Percentage of dose excreted as metabolites (P16D, P18D, 1-OHP)} = K \cdot (1 - e^{-k_{elim} \cdot t}) \quad (1)$$

where K is the asymptotic value and k_{elim} (h^{-1}) is the elimination rate constant.Half-lives ($t_{½}$) were then calculated using:

$$t_{1/2} = \frac{-\ln 2}{k_{elim}} \quad (2)$$

Statistical analysis

Values are reported as mean (SD) values. A Bartlett test was used to test the homogeneity of variances in cumulative urinary excretion values and elimination half-lives of each pyrene metabolite between the different dose groups. One-way analysis of variance (ANOVA) was then performed to test the effect of the dose level on the mean fraction of the dose recovered as P16D, P18D or 1-OHP in the urine of Sprague–Dawley or Wistar rats over the 0–48-h collection period. ANOVA was also performed to test the effect of the dose on the mean urinary excretion rate, hence elimination half-life, of pyrene metabolites. In the case of a difference among the group means, the Tukey post-test was conducted to determine if pairs of mean values were significantly different from each other. Furthermore, an unpaired Student's t-test was conducted to test the difference in both the mean 0–48-h cumulative excretion values (%) and the elimination half-lives of pyrene metabolites between Sprague–Dawley and Wistar rats. The level of significance was set at $p=0.05$.

Results

Figure 2 depicts the urinary excretion rate time courses of P16D, P18D and 1-OHP following an intravenous injection of pyrene in Sprague–Dawley and Wistar rats (values below the limit of detection were not represented on the graphs). P16D and P18D were detectable over the entire collection period (i.e. up to 48-h post-dosing) except at the lowest 0.05 μmol kg^{-1} dose, where excretion values were close to the limit of detection after 24 h. In the case of 1-OHP, excretion values approached the limit of detection between 24- and 48-h post-dosing depending on the dose. Table I also shows the elimination half-lives of pyrene metabolites in both Sprague–Dawley and Wistar rats at the four injected pyrene doses. For a given dose and strain of rats,

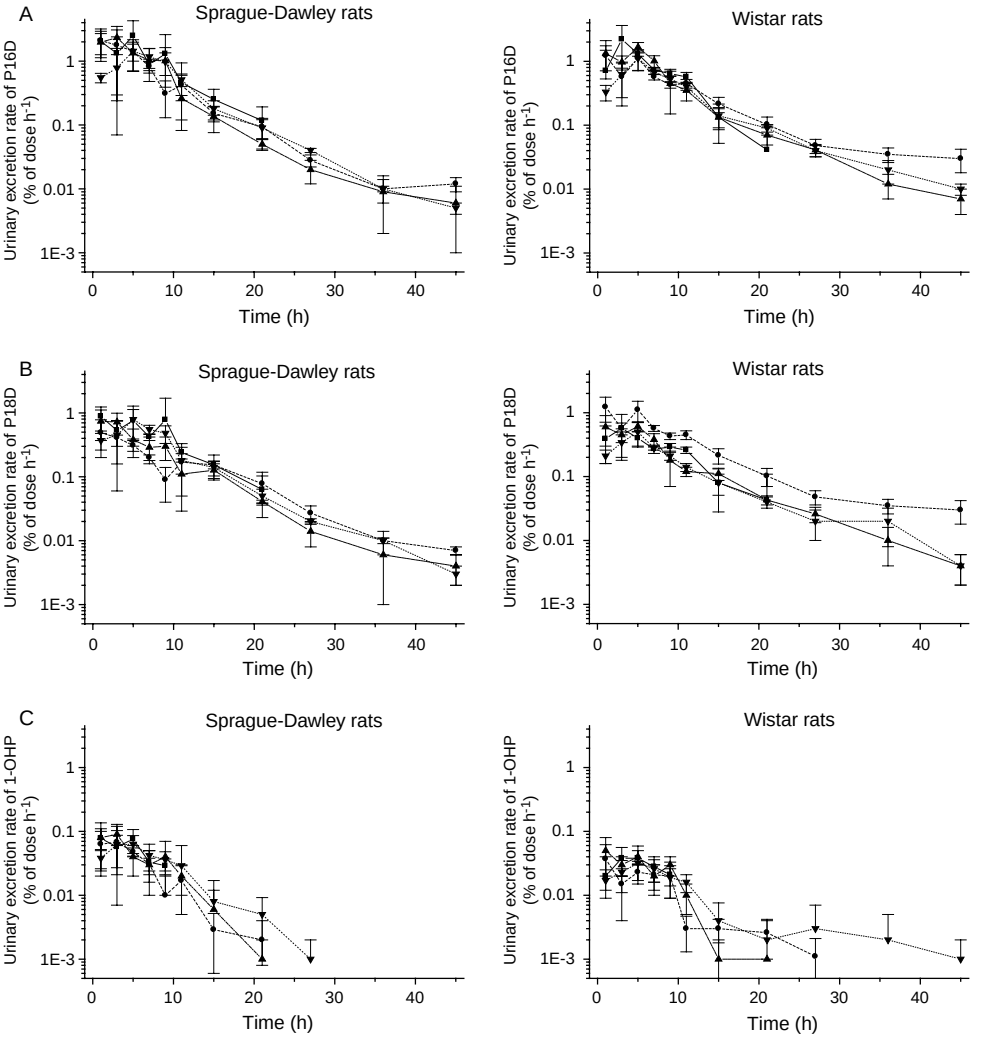

Figure 2. Urinary excretion rate time-courses of pyrene metabolites following an intravenous injection of 0.05 (■), 0.5 (●), 5 (▲) and 50 (▼) μmol kg^{-1} of pyrene in male Sprague–Dawley and Wistar rats. Each point represents mean, and vertical bars are standard deviations ($n = 5$ per group).

excretion curves of P16D, P18D and 1-OHP essentially evolved in parallel; no statistically significant difference in the elimination half-lives was generally observed between the different pyrene metabolites ($p > 0.05$). Furthermore, for a given strain of rats, there was also no clear effect of the increase in the dose on the excretion rate, thus half-life, of the pyrene metabolites except for P16D in Sprague–Dawley rats (Table I). In this latter case, the mean elimination half-life of P16D in Sprague–Dawley rats dosed with 50 μmol kg^{-1} of pyrene was significantly higher than in rats injected with 0.05, 0.5, and 5 μmol kg^{-1} of pyrene ($p < 0.01$). The mean elimination half-life of P16D at the 50 μmol kg^{-1} dose was however not significantly different from that of P18D and 1-OHP ($p > 0.05$).

Table I. Elimination half-life of pyrene-1,6-dione (P16D), pyrene-1,8-dione (P18D) and 1-hydroxypyrene (1-OHP) metabolites in the urine of male Sprague–Dawley and Wistar rats over the 48-h collection period.

	Mean (standard deviation) elimination half-life of pyrene metabolites (h)*					
	Sprague–Dawley rats ($n=5$ per dose)			Wistar rats ($n=5$ per dose)		
Pyrene dose group (μmol kg^{-1})	P16D	P18D	1-OHP	P16D	P18D	1-OHP
0.05	4.3 (0.8)	4.9 (1.2)	3.7 (0.6)	4.5 (1.3)	5.4 (1.0)	3.9 (0.5)
0.5	3.9 (0.6)	6.9 (1.0)	3.6 (0.7)	6.2 (1.1)	7.1 (1.4)	5.2 (1.5)
5	3.9 (0.7)	4.9 (1.0)	3.9 (0.7)	4.7 (0.8)	5.3 (0.5)	3.9 (0.7)
50	5.9 (0.6)	6.0 (0.8)	5.0 (1.2)	6.5 (0.4)	6.6 (0.9)	7.2 (3.5)
Mean†	4.0 (0.7)	5.7 (1.3)	4.1 (0.9)	5.1 (1.3)	6.1 (1.2)	5.1 (2.3)
p‡	<0.01¶	0.02§	0.06	<0.01§	0.02§	0.05

*For each metabolite and strain of rats, the Bartlett test showed all the variances in elimination half-lives were equal between dose groups ($p>0.05$).
†Mean excretion values for a given metabolite were compared between Sprague–Dawley and Wistar rats using a Student's t-test and p values for P16D, P18D and 1-OHP were <0.01 ($n=15$; the highest dose group was excluded given the saturation effect at that dose), 0.29 ($n=20$) and 0.08 ($n=20$), respectively.
‡Comparison of mean excretion values between rats dosed with 0.05, 0.5, 5 and 50 μmol kg^{-1} using the ANOVA test.
¶Tukey post-test showed that the mean excretion value in rats dosed with 50 μmol kg^{-1} was significantly different from those of rats injected with 0.05, 0.5 and 5 μmol kg^{-1}.
§Tukey post-test showed that there was no apparent increase or decrease in the half-life with increasing dose.

Table II describes the cumulative percentage of the pyrene dose excreted as P16D, P18D and 1-OHP in urine following intravenous injection of pyrene, at four different doses, in Sprague–Dawley and Wistar rats. In both strains, the P16D and P18D metabolites were excreted in urine in significantly higher percentage than 1-OHP, whatever the dose. Excretion values of P16D and P18D were on average 23–46 and 10–17 times higher, respectively, than those of 1-OHP. 1-OHP was also subject to more inter-individual variations than P16D and P18D; from Table II, the coefficient of variation (CV) was calculated to be 28% on average for 1-OHP as compared with 8.5% for P16D and 16% for P18D. Table II also shows that for 1-OHP, there was no significant effect of the dose on the 0–48h cumulative excretion of 1-OHP ($p>0.05$) in Sprague–Dawley and Wistar rats. However, a statistically significant difference in the 0–48-h cumulative percentage of dose excreted as P16D according to the dose was observed in both strains of rats ($p<0.01$). More specifically, in Sprague–Dawley rats, total urinary excretion of P16D was significantly lower at the 50 μmol kg^{-1} dose than at the 0.05, 0.5 and 5 μmol kg^{-1} doses; in Wistar rats, a significant difference in total P16D excretion at the 50 μmol kg^{-1} dose as compared with the 0.05 and 5 μmol kg^{-1} doses was noted. For P18D, there was no clear effect of the increase in pyrene dose on its overall excretion in urine during the 48-h period post-treatment.

Inter-strain comparison of the cumulative percentage of the pyrene dose excreted as pyrene metabolites in urine showed that Sprague–Dawley rats excreted more P16D, P18D and 1-OHP in urine than Wistar rats ($p<0.01$) (Table II). Table I also shows that P16D exhibited a significantly slower elimination in Wistar rats than in Sprague–

Table II. Percentage of the injected pyrene dose recovered as pyrene-1,6-dione (P16D), pyrene-1,8-dione (P18D) and 1-hydroxypyrene (1-OHP) metabolites in the urine of male Sprague–Dawley and Wistar rats over the 48-h collection period.

	Mean percentage of the injected pyrene excreted overall as metabolites (standard deviation)*					
	Sprague–Dawley rats ($n=5$ per dose)			Wistar rats ($n=5$ per dose)		
Pyrene dose group (μmol kg^{-1})	P16D	P18D	1-OHP	P16D	P18D	1-OHP
0.05	25.6 (2.4)	8.2 (2.7)	0.7 (0.2)	14.6 (1.8)	5.5 (0.9)	0.3 (0.1)
0.5	21.6 (1.8)	6.4 (0.8)	0.6 (0.1)	12.9 (0.8)	4.8 (0.5)	0.3 (0.1)
5	24.0 (2.1)	8.0 (1.4)	0.8 (0.2)	14.7 (2.2)	6.4 (1.1)	0.4 (0.1)
50	17.4 (1.4)	8.8 (0.7)	0.8 (0.3)	10.3 (1.3)	4.9 (0.6)	0.4 (0.2)
Mean[†]	23.7 (2.6)	7.8 (1.7)	0.7 (0.2)	14.1 (1.8)	5.4 (1.0)	0.4 (0.1)
p value[‡]	<0.01[¶]	0.15	0.42	< 0.01[§]	0.02[§]	0.53

*The Bartlett test showed all variances in the percentage of dose excreted as a given pyrene metabolite in a strain of rats were homogeneous between dose groups ($p > 0.05$).

[†]Mean excretion values for a given metabolite were compared between Sprague–Dawley and Wistar rats using a Student's t-test and p values were <0.01 for all metabolites (for P16D, the highest dose group was excluded given the saturation effect at that dose).

[‡]Comparison of mean excretion values between rats dosed with 0.05, 0.5, 5 and 50 μmol kg^{-1} using the ANOVA test.

[¶]Tukey post-test showed that the mean excretion value in rats dosed with 50 μmol kg^{-1} was significantly different from those of rats injected with 0.05, 0.5 and 5 μmol kg^{-1}.

[§]Tukey post-test showed that there was no apparent increase or decrease in the percentage of pyrene dose excreted as P18D with increasing dose.

Dawley rats ($p < 0.01$). The urinary excretion rate of 1-OHP and P18D also tended to be lower in Wistar rats although the difference was not significant.

Discussion

This study was designed to document the urinary excretion time courses of the dioxygenated pyrene metabolites in comparison with that of 1-OHP following different exposure doses in two strains of rats. It was intended to assess the potential usefulness of these metabolites as bioindicators of exposure to pyrene. Sprague–Dawley and Wistar rat strains were used since they are the most employed in PAH metabolism research, but they had not yet been compared in the same study. The intravenous administration route was chosen as a reference route to avoid bioavailability, absorption rate or first-pass effects on the kinetics.

In general, it was observed that the urinary excretion time course of the dioxygenated pyrene metabolites was similar to that of 1-OHP but pyrene diones were much more abundant metabolites in urine than 1-OHP over the dose range tested (23–46 times for P16D and 10–17 times for 18D). P16D and P18D excretion was also subject to less inter-individual variations than 1-OHP excretion. However, the analytical limits of detection for P16D and P18D were respectively 34 and 18 times less than that of 1-OHP under the derivatization conditions used (Ruzgyte et al. 2005). In a previous study in rats injected intravenously with ^{14}C-labelled pyrene, it

was also shown that 1-OHP was not the major pyrene metabolites found in urine but that the urinary excretion time course of ^{14}C-pyrene equivalents, hence total pyrene metabolites followed that of 1-OHP (Bouchard et al. 1998a). In this latter study, metabolites other than 1-OHP were, however, not identified specifically.

In the current study, a strong linear dose–excretion relationship was also observed for 1-OHP and P18D over the 0.05–50 μmol kg^{-1} dose range. This is in accordance with previous findings showing that the cumulative amounts of 1-OHP excreted in urine were proportional to the dose in rats intravenously injected with 0.5–50 μmol kg^{-1} of pyrene (Bouchard & Viau 1996). As for P16D excretion, it was linear over the 0.05–5 μmol kg^{-1} dose range, hence over two orders of magnitude, but at the highest 50 μmol kg^{-1} dose, the percentage of the pyrene dose excreted overall as P16D was statistically lower than at the other doses. More experimental work is however needed to verify the possible non-linear trend at high doses (> 5 μmol kg^{-1}). Withey et al. (1991) reported that the intravenous injection of 2, 4, 6, 9 and 15 mg of ^{14}C-pyrene kg^{-1} body weight^{-1} (10, 20, 30, 44.5 and 74 μmol kg^{-1}) produced a constant mean cumulative percentage of total ^{14}C-pyrene equivalents recovered in Wistar rat urine at different time points (0.5, 1, 2, 4 and 6 days). These authors, however, also established the blood concentration–time course of ^{14}C-equivalents and pyrene in that study. A two-compartment model with first-order elimination was fitted to the latter data; the initial elimination phase represented the combined distribution of pyrene to storage tissues and elimination of pyrene from blood and tissues in equilibrium with blood through biotransformation while the terminal phase represented elimination of pyrene from storage tissues. Although the terminal elimination phase was parallel across dose, the elimination rate of the initial phase tended to be slower at the two highest doses suggesting a saturable biological process at these doses, plausibly a saturation of the metabolism of pyrene.

It should, however, be stressed that the highest dose used in the current study (50 μmol kg^{-1}) corresponds to a human equivalent dose (HED) of about three orders of magnitude higher than the expected daily dose of a heavily exposed worker. Indeed, if one considers the new default procedure used by the US Environmental Protection Agency (EPA) to derive a HED from animal data, the 50 μmol kg^{-1} dose administered in rats corresponds to a HED of about 12 μmol kg^{-1} or 2.3 mg kg^{-1} (EPA 2000). By comparison, the daily pyrene exposure dose of a worker exposed at airborne PAH levels corresponding to the threshold limit value (TLV) of 200 μg m^{-3} for coal tar pitch volatiles is expected to be around 3 μg kg^{-1} or 14 nmol kg^{-1}, considering that pyrene represents approximately 10% of PAH mixtures (200 μg m^{-3} × 10 m^3 day^{-1}/70 kg) × 10% ≈ 3 μg kg^{-1} or 14 nmol kg^{-1}).

In the present study, inter-strain differences in the kinetics of pyrene metabolites were also observed. The mean cumulative urinary excretion of the sum of P16D, P18D and 1-OHP amounted to approximately 27–34% of the pyrene dose for Sprague–Dawley rats and 16–21% for Wistar rats over the 48-h collection period (Table II). The ratio between Sprague–Dawley and Wistar rats is in agreement with previous findings showing that on average 57% of an intravenous ^{14}C-pyrene dose of 50 μmol kg^{-1} was recovered as total ^{14}C-pyrene equivalents in the urine of Sprague–Dawley rats during the 24-h period post-dosing (Bouchard et al. 1998a) as compared with on average 29–40%, depending on the dose, in Wistar rats treated with 10–74 μmol kg^{-1} of pyrene (Withey et al. 1991). During that period of time, on average 25% of the intravenous pyrene dose was recovered in the gastrointestinal tract and faeces of the Sprague–Dawley rats while 32–49% was excreted in faeces of Wistar rats. These results suggest that the inter-strain

variations could be explained by a lower biliary excretion in Sprague–Dawley rats than in Wistar rats or by differences in the enterohepatic recycling but this remains to be elucidated (Withey et al. 1991, Bouchard et al. 1998a). Sprague–Dawley rats may also have a higher capacity for oxidative metabolism of pyrene compared with Wistar rats.

With regard to the urinary excretion rate of the major pyrene metabolite, P16D, it was slightly faster in Sprague–Dawley rats than in Wistar rats (Table I). P18D and 1-OHP also tended to be excreted more rapidly in Sprague–Dawley rats but the difference was not statistically significant. In previous studies, the elimination time course of pyrene in blood was assessed in Sprague–Dawley (Bouchard et al. 1998a) and Wistar rats (Withey et al. 1991) by fitting to a two-compartment model with first-order elimination. The elimination rate of pyrene from blood was 0.012 min^{-1} in both Sprague–Dawley and Wistar rats administered similar doses (50 and 44.5 μmol kg^{-1}, respectively). This suggests that pyrene is eliminated through biotransformation at a similar rate in both strains of rats at this dose. In a previous study in Sprague–Dawley rats, it has moreover been shown that, compared with renal clearance of the metabolites, biotransformation of pyrene is very rapid and is not a rate-limiting factor in the urinary excretion rate of 1-OHP (Bouchard & Viau 1996). This has been evidenced by the similar urinary excretion courses of 1-OHP following intravenous injection of either pyrene or the metabolite itself. The observed inter-strain differences in the urinary excretion rate of the major pyrene metabolite could therefore more plausibly stem from variations in the elimination rate of the metabolite itself, such as differences in the enterohepatic recycling (Bouchard et al. 1998a, Viau et al. 1999).

This study suggests that pyrene diones could be good biomarkers of exposure to pyrene in complement with 1-OHP. Biomonitoring studies in human populations exposed to PAHs should be conducted to assess the potential usefulness of these biomarkers.

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