



Title	Comparison of xenobiotic metabolism in phase I oxidation and phase II conjugation between rats and bird species
Author(s)	Saengtienchai, Aksorn; Ikenaka, Yoshinori; Kawata, Minami; Kawai, Yusuke; Takeda, Kazuki; Kondo, Takamitsu; Bortey-Sam, Nesta; Nakayama, Shouta M. M.; Mizukawa, Hazuki; Ishizuka, Mayumi
Citation	Comparative biochemistry and physiology Part C: Toxicology & pharmacology, 214, 28-35 https://doi.org/10.1016/j.cbpc.2018.08.007
Issue Date	2018-12
Doc URL	http://hdl.handle.net/2115/76244
Rights	© 2018. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/
Rights(URL)	https://creativecommons.org/licenses/by-nc-nd/4.0/
Type	article (author version)
File Information	Comparative biochemistry and physiology Part C_214_28_35.pdf



[Instructions for use](#)

1 Comparison of xenobiotic metabolism in phase I oxidation and phase II conjugation between rats
2 and bird species

3

4 Aksorn Saengtienchai^{a,b}, Yoshinori Ikenaka^{*a,c}, Minami Kawata^a, Yusuke Kawai^d, Kazuki
5 Takeda^a, Takamitsu Kondo^a, Nesta Bortey-Sam^a, Shouta M.M. Nakayama^a, Hazuki Mizukawa^e,
6 Mayumi Ishizuka^a

7

8 ^aLaboratory of Toxicology, Department of Environmental Veterinary Sciences, Graduate School
9 of Veterinary Medicine, Hokkaido University, N18 W9, Kita-ku, Sapporo 060-0818, Japan

10 ^bDepartment of Pharmacology, Faculty of Veterinary Medicine, Kasetsart University, 50 Ngam
11 Wong Wan Rd, Lat Yao, Chatuchak, Bangkok 10900, Thailand

12 ^cWater Research Group, Unit for Environmental Sciences and Management, North-West
13 University, Potchefstroom, South Africa

14 ^dDepartment of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary
15 Medicine, Inada-cho, Obihiro, Hokkaido, 080-8555, Japan

16 ^eDepartment of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine,
17 Hokkaido University, N18 W9, Kita-ku, Sapporo 060-0818, Japan

18

19 *Corresponding Author

20 Yoshinori Ikenaka

21 E-mail: y_ikenaka@vetmed.hokudai.ac.jp

22 TEL: +81-11-706-5102

23

24

25

26 **ABSTRACT**

27

28 There have been many reports regarding toxic chemicals in birds. Chemicals are mainly
29 metabolized in the liver through phase I oxidation by cytochrome P450 (CYP) and phase II
30 conjugation by conjugated enzymes, such as UDP-glucuronosyltransferase (UGT),
31 sulfotransferase (SULT), glutathione-S-transferase (GST), etc. Xenobiotic metabolism differs
32 among bird species, but little detailed information is available. In the present study, the four-ring
33 polycyclic aromatic hydrocarbon (PAH), pyrene, was used as a model xenobiotic to clarify the
34 characteristics of xenobiotic metabolism in birds compared with laboratory animals by *in vivo*
35 and *in vitro* studies. Plasma, bile, and excreta (urine and feces) were collected after oral
36 administration of pyrene and analyzed to clarify xenobiotic metabolism ability in chickens and
37 quails. Interestingly, pyrenediol-glucuronide sulfate (PYDOGS) and pyrenediol-diglucuronide
38 (PYDOGG) were present in chickens and quails but not in rats. In addition, the area under the
39 curve (AUC), maximum plasma concentration (C_{max}), and time to maximum plasma
40 concentration (T_{max}) of pyrene-1-sulfate (PYOS) were higher than those of the parent molecule,
41 pyrene, while the elimination half-life ($t_{1/2}$) and mean residence time (MRT) were faster than
42 those of the parent pyrene. With regard to sulfation of 1-hydroxypyrene (PYOH), the maximum
43 velocity (V_{max}) and Michaelis constant (K_m) of rat liver cytosol were greater than those of
44 chicken and quail liver cytosol. Furthermore, V_{max}/K_m of UGT activity in rat liver microsomes
45 was also greater than those of chicken and quail liver microsomes. Characterization of xenobiotic
46 metabolism revealed species differences between birds and mammals, raising concerns about
47 exposure to various xenobiotics in the environment.

48

49 **Keywords:** birds, species differences, pyrene, conjugated metabolites, kinetics

50

51

52 1. Introduction

53

54 Since the 1950s, there have been increasing reports of injuries to wild birds worldwide due to
55 the influence of various xenobiotics, such as dichlorodiphenyltrichloroethane (DDT), coumarin-
56 derived anticoagulant rodenticides, and non-steroidal anti-inflammation drugs (NSAIDs), such as
57 diclofenac (Bowerman et al., 1995; Elliott et al., 1988; Erickson et al., 2004; Norstrom and
58 Hebert, 2006; Prakash et al., 2007). These xenobiotics cause secondary poisoning that could
59 affect and damage the reproductive system, liver, and kidney in scavenging and raptorial birds
60 (Albers et al., 2003; Albert et al., 2010; Erickson et al., 2004; Prakash et al., 2007).

61 Polycyclic aromatic hydrocarbons (PAHs) are a ubiquitous group of several chemically
62 related compounds produced naturally and by human activities. They also persist in the
63 environment and show varied toxic effects on organisms through various actions causing
64 carcinogenic and mutagenic effects. Generally, PAHs enter the environment and animal bodies
65 through various routes, and are usually found as mixtures containing two or more of these
66 compounds (Armstrong et al., 2004). In birds, the mechanism of toxicity is considered to involve
67 interference with the function of cellular membranes as well as with enzyme systems associated
68 with the cell membrane. Toxic effects have been documented mostly in embryos, young birds,
69 and adult birds. There have also been reports of reduced egg production, hatching, and growth
70 (Albers et al., 2003). Although PAHs influence absorption to organic material or degradation in
71 the environment, some are persistent and bioaccumulate in the food chain (Jiang et al., 2011).
72 Wild and domestic birds may be exposed to PAHs and accumulate them in their bodies.
73 Xenobiotics, including PAHs, are generally absorbed and distributed in the body, and are
74 metabolized based by phase I oxidation (mainly enzymes as cytochrome P450s) and phase II
75 conjugation enzymes, such as UDP-glucuronosyltransferase (UGT), sulfotransferase (SULT),
76 and glutathione-S-transferase (GST). The more water-soluble metabolites are excreted in the
77 urine and feces. However, there have been reports of interspecies differences in xenobiotic
78 metabolic activity among bird species *in vitro* (Watanabe et al., 2010). However, insufficient
79 information is available to clarify the xenobiotic metabolism ability in phase I oxidation and
80 phase II conjugation in bird species *in vivo*, especially with regard to their kinetic parameters.

81 Pyrene, a four-ring PAH, was selected as a xenobiotic model to observe the metabolic
82 activity in birds in comparison with laboratory animals following *in vivo* and *in vitro* studies. In
83 addition, pyrene and its metabolites are typical phenolic xenobiotic models. To understand
84 xenobiotic metabolism in bird species, we used pyrene as a xenobiotic model and analyzed
85 pyrene metabolites in urine. The excretion of urine containing pyrene metabolites is useful to
86 characterize differences in phase II xenobiotic conjugation reactions between species
87 (Saengtienchai et al., 2016).

88 To clarify the roles of phase I oxidation and phase II conjugation reactions of pyrene in bird
89 species in comparison with laboratory animals, an *in vivo* exposure study was performed. An
90 important factor in pharmacokinetics of pyrene and its conjugated metabolites were observed to
91 complete the absorption, distribution, metabolism, and excretion in birds. To assess the
92 efficiency of phase II conjugation enzymes *in vitro*, 1-hydroxypyrene (PYOH) was chosen as a
93 substrate to measure UGT-dependent and SULT-dependent activities.

94 2. Materials and methods

95

96 2.1. Animals

97 Nine-week-old male Wistar rats (*Rattus norvegicus*) ($n=3$) were obtained from SLP
98 (Hamamatsu, Japan). Eight-week-old male White Leghorn chickens (*Gallus gallus*) ($n=3$) were
99 obtained from Hokudo Co., Ltd. (Sapporo, Japan). One-year-old quails (*Coturnix japonica*)
100 (male, $n=1$; female, $n=2$) were obtained from a local commercial supplier in Sapporo, Japan.
101 The animals were acclimated for one week in the laboratory and kept under conditions of 40%–
102 70% humidity at $23\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ in a temperature-controlled room with a 12-h light/dark cycle.
103 They were given laboratory food and clean water *ad libitum*. All animal experiments were
104 performed under supervision and with the approval of the Institutional Animal Care and Use
105 Committee of Hokkaido University (approval no. 100067), in accordance with the Association
106 for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

107

108 2.2. Chemicals

109 Pyrene, PYOH, 3'-phosphoadenosine 5'-phosphoslfate (PAPS), sulfatase (from limpet Type
110 V; 34 U/mg), β -glucuronidase (from bovine liver, Type B-1; 1,240 U/mg), β -glucosidase (from
111 almond; 3.4 U/mg), and bovine serum albumin were obtained from Sigma-Aldrich Co. (St.
112 Louis, MO). Methanol, acetonitrile, acetic acid, and ammonium acetate were purchased from
113 Kanto Chemical Co., Inc. (Tokyo, Japan). Pyrene-1-sulfate (PYOS) was obtained from TOPU
114 Bio (Toyama, Japan). Uridine-diphosphate-glucuronic acid (UDP-GA) was purchased from
115 Wako (Osaka, Japan). All chemicals used for high-performance liquid chromatography (HPLC)
116 and mass spectrometry (MS) were of HPLC or MS grade, respectively, and were obtained from
117 Kanto Chemical Co. Inc.

118

119 2.3. Pyrene exposure and sample collection

120 Chickens, quails, and rats were fasted for 24 h before exposure to pyrene as the test chemical.
121 Pyrene was dissolved in corn oil and administered orally at a dose of 4 mg/kg body weight.

122 Plasma was collected from chickens at 0, 1, 2, 3, 6, 9, 12, 24, and 48 h after pyrene exposure for
123 pharmacokinetic analysis. In rats, plasma samples were collected at 24 h after oral administration
124 of pyrene. In the case of quails, plasma samples were collected after 2 h exposure, which showed
125 a high distribution of pyrene metabolites. The excreta, such as urine, feces, urine-feces mixed (in
126 chickens and quails), bile (in chickens), and urine contained in the ureter (in chickens) were
127 obtained from chickens, quails, and rats after 24 h of pyrene exposure. Samples were kept at –
128 20 °C until analysis. Animals were then sacrificed by carbon dioxide inhalation at 48 h after
129 pyrene exposure. The livers were removed and perfused with cold 1.15% potassium chloride to
130 remove blood, and samples were immediately placed in liquid nitrogen and kept at –80 °C.

131

132 *2.4. Extraction of pyrene metabolites in samples from in vivo study*

133 The method of Boocook et al. (2007) was used in this study. Briefly, aliquots of 10 µL of
134 plasma from each animal species were extracted with 90 µL of 100% methanol. The mixed
135 samples were stored at –20 °C for 50 min to precipitate protein. After melting at room
136 temperature, samples were centrifuged at 13,000g for 15 min at room temperature. Aliquots of
137 10 µL of the supernatant were subjected to HPLC with fluorescence detection (FD).

138 Urine and feces samples were extracted and cleaned up according to the protocol described
139 by Saengtienchai et al. (2015). Briefly, 1 mL of urine was extracted with the same amount of
140 70% methanol (50:50, v:v). The mixtures were extracted by vortexing for 1 min and
141 centrifugation at 9,000g for 10 min at room temperature. The supernatants were then filtered
142 with a 0.2-µm syringe filter (SupraPure; Recenttec, Tokyo, Japan). Aliquots of 1 µL of the
143 filtrates were subjected to HPLC/FD.

144 Samples of approximately 1 g of feces or urine-feces mixture were homogenized with 20 mL
145 of 70% methanol. The mixtures were then extracted by sonication for 20 min and centrifuged at
146 9,000g for 10 min at room temperature. The supernatants were transferred into 50-mL Falcon
147 tubes. The residual samples were extracted again. The pooled supernatants were filtered with a
148 0.2-µm syringe filter and kept at –20 °C until analysis. Then, 5 µL of each pooled supernatant
149 was subjected to HPLC/FD.

150 Aliquots of bile samples were diluted 400-fold with 70% methanol. The samples were then
151 centrifuged at 10,000g for 5 min at room temperature. Then 5 μ L of each supernatant was
152 subjected to HPLC/FD.

153 The urine contained in the ureter samples were extracted with 500 μ L of 70% methanol. The
154 mixtures were then extracted by sonication for 20 min and centrifuged at 10,000g for 5 min at
155 room temperature. Then, 5 μ L of each supernatant was analyzed by HPLC/FD.

156

157 *2.5. Analysis and identification of pyrene metabolites*

158 The supernatant was then injected into the HPLC/FD system (pump: LC-20AD, auto
159 sampler: SIL-20A, column oven: CTO-20A, controller: CEM-20A; Shimadzu) equipped with an
160 ODS column (ODS-120T 4.6 mm \times 300 mm; Tosoh, Tokyo, Japan). HPLC was performed
161 according to the method of Beach et al. (2010) with slight modifications. Mobile phase A
162 consisted of a mixture of 10 mM ammonium acetate buffer (pH 5) and mobile phase B (9:1, v/v).
163 A mixture of methanol:acetonitrile:water (38:57:5, v/v/v) was used as mobile phase B. The
164 solvent gradient was 100% mobile phase A at 0 min, followed by a linear gradient to 100% of
165 mobile phase B from 0 to 35 min. The gradient was held at 100% of mobile phase B for 5 min
166 until 40 min. Then, the gradient was held at 100% of mobile phase A from 40.1 to 47 min. The
167 solvent flow rate was set at 0.5 mL/min, and a column temperature of 45 $^{\circ}$ C was used
168 throughout. The excitation and emission wavelengths for fluorescence detection were 343 and
169 385 nm, respectively. Furthermore, each separated peak was identified by electrospray ionization
170 ion-trap mass spectrometry (ESI/ion-trap/MS, LTQ XL; Thermo Fisher Scientific, Waltham,
171 MA). The ESI conditions were fully scanned (m/z 80–800) in negative mode, with an ion source
172 voltage and temperature of -4.2 kV and 420 $^{\circ}$ C, respectively.

173

174 *2.6. Deconjugation of pyrene metabolites*

175 Deconjugation was performed using the method described by Ikenaka et al. (2007).
176 Briefly, the enzymes sulfatase, β -glucuronidase, and β -glucosidase were dissolved in 0.1 M
177 sodium acetate buffer and the pH was adjusted to 5.0 with acetic acid. The enzyme

178 concentrations were 10, 4000, and 17 U/mL, respectively. Aliquots of 30 μ L of urine sample
179 extracts containing pyrene metabolites were mixed with 270 μ L of buffer, and each
180 deconjugation enzyme (200 μ L) was added. As a control, the same quantity of bovine serum
181 albumin (1 mg/mL) was added, and reactions were performed under the same conditions as used
182 for the deconjugation enzymes. All samples were incubated at 37 °C for 8 h. The reaction was
183 stopped by adding 500 μ L of methanol. The deconjugation solutions were analyzed by
184 HPLC/FD.

185

186 *2.7. Preparation of liver microsomal and cytosolic fractions*

187 Liver microsomal and cytosolic fractions were prepared according to the method of
188 Omura and Sato (1964). The liver samples were homogenized in potassium phosphate buffer
189 (KPB; 0.1 M, pH 7.4) on ice. The homogenates were transferred to tubes and centrifuged at
190 9,000g for 20 min at 4 °C. The supernatants (S9 fraction) were decanted into ultracentrifugation
191 tubes and centrifuged at 105,000g, 4 °C for 70 min. Each homogenate consisted of two parts,
192 with the supernatant containing the cytosolic fraction. The pellets were homogenized on ice with
193 KPB and washed by centrifugation at 105,000g, 4 °C for 70 min. The microsomal pellets were
194 homogenized with KPB again. The microsomal and cytosolic fractions were transferred to 1.5-
195 mL tubes and stored at -80 °C. The cytosolic fractions were used to measure SULT activity;
196 microsomal fractions were used to determine UGT activities. The protein concentrations in the
197 microsomes and cytosol were determined using the method of Lowry et al. (1951).

198

199 *2.8. UGT-dependent PYOH glucuronidation activity*

200 UGT activity of PYOH was assessed using the method described by Ueda et al. (2011)
201 with slight modifications. Initially, microsomes were diluted to 4 mg/mL protein with 0.1 M
202 phosphate buffer (pH 7.4), and then 2.5 μ L of 1% sodium cholate solution was added and
203 incubated on ice for 30 min. After treatment, 50 μ L of microsome solution was mixed with 41.5
204 μ L of buffer, 5 μ L of 100 mM MgCl₂, and 1 μ L of PYOH. The final concentrations of PYOH
205 were 10, 20, 25, 38, 50, 100, and 200 μ M. Samples were preincubated at 37 °C for 5 min. The
206 reaction was started with 2.5 μ L of a 50 mM UDP-GA in a final volume of 100 μ L. The reaction

207 was allowed to proceed for 10 min, and 400 μ L of ice-cold methanol was added to stop the
208 reaction. The reaction solutions were placed on ice for 20 min, and then centrifuged at 1,600g for
209 15 min. The supernatants were transferred to HPLC vials and subjected to HPLC/FD
210 (Shimadzu). Samples were analyzed with an Inertsil ODS-3 column (2.1 mm \times 150 mm; GL
211 Sciences). Mobile phase A was 10 mM ammonium acetate buffer (pH 5) and mobile phase B
212 consisted of methanol:acetonitrile:water (38:57:5, v/v/v). The linear gradient was 17 min in
213 length with 90% phase B. injection volume was 10 μ L, the flow rate was 0.5 mL/min, and the
214 column temperature was 45 $^{\circ}$ C. Excitation and emission wavelengths were 343 and 385 nm,
215 respectively. Kinetic parameters, including maximal velocity (V_{\max}), Michaelis–Menten constant
216 (K_m), and V_{\max}/K_m ratio (enzyme efficiency), were determined by the Michaelis–Menten
217 equation using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA).

218

219 2.9. *SULT-dependent PYOH sulfation activity*

220 The SULT activity for PYOH was determined using a modification of the method of
221 Ueda et al. (2011). The final protein concentration in the liver cytosol was 250 μ g protein/mL,
222 which was adjusted with 100 mM Tris-HCl buffer (pH 7.4). Then, 50 μ L of cytosol solution was
223 mixed with 10 μ L of 100 mM MgCl₂, 10 μ L of 50 mM Na₂SO₃, and 1 μ L of 1-hydroxypyrene.
224 Tris-HCl buffer (100 mM, pH 7.4) was added to make up the volume to 97.5 μ L. The final
225 concentrations of 1-hydroxypyrene were 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, and 0.5 μ M. The
226 mixtures were preincubated at 37 $^{\circ}$ C for 5 min. The reaction was initiated by adding 2.5 μ L of 1
227 mM PAPS and the final volume was 100 μ L. After 10 min of incubation, 400 μ L of ice-cold
228 methanol was added to stop the reaction. The samples were placed on ice for 20 min, and then
229 centrifuged at 1,600g for 15 min. The supernatants were transferred into HPLC vials and
230 subjected to HPLC/FD (Shimadzu). Samples were analyzed with an Inertsil ODS-3 column (2.1
231 mm \times 150 mm: GL Sciences, Inc.). Mobile phase A was 10 mM ammonium acetate buffer (pH 5)
232 and mobile phase B consisted of methanol:acetonitrile:water (38:57:5, v/v/v). The linear gradient
233 was 17 min with 90% phase B. Injection volume was 10 μ L, the flow rate was 0.5 mL/min, and
234 the column temperature was 45 $^{\circ}$ C. Excitation and emission wavelengths were 343 and 385 nm,
235 respectively. Kinetic parameters were determined using the Michaelis–Menten equation, as
236 described above.

237

238 *2.10. Statistical analysis*

239 The results were subjected to Tukey's HSD test (JMP 7.0; SAS Institute Inc., Raleigh,
240 NC). All kinetic parameters (Michaelis–Menten equation) were analyzed using GraphPad Prism
241 Version 5.01. In all analyses, $p < 0.05$ was taken to indicate statistical significance.

242

243 3. Results

244

245 3.1. Identification of pyrene metabolites in chicken

246 The chromatograms of pyrene metabolites in chicken bile are shown in Fig. 1. A total of six
247 pyrene metabolites were detected, peak-a to peak-f, with retention times (RT) of 11.6, 14.8, 15.0,
248 17.4, 22.9, and 25.9 min, respectively. Table 1 presents a summary of each RT, results of
249 deconjugation enzymes treatment, and estimated metabolites. PYOH and pyrene were eluted at
250 RT of 36 and 41.8 min, respectively. The other unidentified peaks were determined by
251 LC/MS/MS. The ESI negative mass spectra of peak-a (RT 11.6 min) had a parent ion of mass to
252 charge ratio (m/z) 489 (MS) and MS² of m/z 409 (M-80; considered as sulfate moiety), 313 (M-
253 176; glucuronide moiety), and 233 (M-256; both sulfate and glucuronide moieties). Peak-b
254 eluted at 14.8 min and contained a major ion at m/z 585 (MS) with MS² of m/z 409 (M-176;
255 glucuronide moiety). Peak-c was eluted at RT 15.0 min and had m/z 393 (MS) including MS² of
256 m/z 313 (M-80; sulfate moiety) and 233 (M-160; di-sulfate moieties). The metabolite from peak-
257 d (RT 17.4 min) consisted of an ion at m/z 313 (MS) and MS² at m/z 233 (M-80; sulfate moiety).
258 Peak-e eluted at 22.9 min with parent ion at m/z 393 (MS) and MS² at m/z 217 (M-176;
259 glucuronide moiety) it was pyrene-1-glucuronide (PYOG). Furthermore, peak-f was eluted at
260 25.9 min with m/z of 297 (MS) and MS² 217 (M-80; sulfate moiety) and it was suspected to be
261 PYOS. These results suggested that pyrenediol (PYDOH) (m/z 233) could be a conjugation
262 product of peak-a, c, and d. In addition, peak-e and f also had PYOH (m/z 217) as a conjugation
263 product. Interestingly, the conjugation product of peak-b seemed to differ from the other peaks.
264 The ion of m/z 409 may have been similar to PYDOG.

265 Deconjugation conditions were used to identify each pyrene metabolite peak (peak-a to peak-
266 f) in chicken bile after collection of each peak by fraction collectors. The fractions were treated
267 with the specific enzymes used to digest the conjugated metabolites. Sulfatase, β -glucuronidase,
268 and β -glucosidase were used for deconjugation (Table 1). Peak-a, c, d, and f disappeared after
269 sulfatase treatment. In the case of β -glucuronidase treatment, peak-a disappeared completely,
270 while this enzyme reduced peak-e by about 10%. However, all six peaks did not respond to β -
271 glucosidase treatment. These results indicated that peak-a to peak-f were pyrene conjugated
272 metabolites in chicken bile. Peak-a was suspected to be a PYDOGS, peak-b to be a PYDOGG,

273 peak-c to be a PYDOSS, peak-d to be a PYDOS, peak-e, to be a PYOG, and peak-f , to be a
274 PYOS.

275

276 *3.2. Qualification and distribution ratio of pyrene metabolites in chickens, quails, and rats*

277 Chickens, quails, and rats were exposed to pyrene, and plasma was collected at 24 h after
278 oral administration. In chicken and quail plasma, the conjugated metabolites detected were
279 PYDOGS, PYDOGG, PYDOSS, PYDOS, PYOG, and PYOS (Fig. 2). Although these three-
280 species had similar types of conjugated pyrene metabolites, the composition of pyrene
281 metabolites which are derived from PYDOH (such as PYDOSS and PYDOS) were seemed to be
282 higher in rat plasma than that in chicken and quail. On the other hand, more than 90% of pyrene
283 metabolites in both chicken and quail plasma were PYOS, while a smaller proportion of
284 glucuronide conjugation was detected. Interestingly, sulfate conjugates were the main
285 metabolites present in plasma of both bird species and rats. In addition, only PYDOGG and
286 PYDOGS were detected in bird plasma but not in rat plasma.

287 The type of conjugated metabolites in the excreta (urine and feces) in both chicken and quail,
288 such as PYDOSS, PYDOS, PYDOGS, PYDOGG, PYOS, and PYOG, were similar as observed
289 in plasma. On the other hand, the proportion of pyrene metabolites between plasma and excreta
290 in chicken and quail were different (Fig. 2). Although more than 90% of the conjugated
291 metabolites were found as PYOS in plasma, various types of the metabolites were equally
292 detected in excreta. Interestingly, only PYOH was present in rat feces.

293 Moreover, bile and urine in the ureter from chickens were collected to examine pyrene
294 metabolites. The results are summarized in Fig. 2. These samples showed similar conjugated
295 pyrene metabolites present as sulfate and glucuronide conjugates. Only PYOH was present in
296 bird excreta but not in plasma, bile, or urine contained in the ureter. The order for percentages of
297 PYDOSS and PYDOS was as follows: excreta>urine contained in the ureter>bile and plasma.
298 PYOS was mainly present in chicken samples in the order: plasma>urine contained in the ureter
299 >bile and excreta. In addition, PYDOS, PYDOGG, and PYOG were detected at high levels in
300 bile, excreta, urine contained in the ureter, and plasma, respectively.

301

302 *3.3. Pharmacokinetic evaluation in chickens*

303 The distributions of each peak area of pyrene metabolite-time profiles of pyrene following a
304 single oral administration are shown in Fig. 3. Both glucuronide and sulfate conjugated
305 metabolites were detected, and PYOS was the dominant metabolite among all conjugated pyrene
306 metabolites. Corresponding exposure data in terms of area under the curve (AUC), maximum
307 plasma concentration (C_{\max}), time to maximum plasma concentration (T_{\max}), elimination half-life
308 ($t_{1/2}$), mean residence time (MRT), and total clearance of the drug from plasma after oral
309 administration (CL/F) of pyrene and its metabolite, PYOS, in chicken plasma are summarized in
310 Table 2. Pyrene was rapidly absorbed after oral administration, the peak concentration occurring
311 faster than 1 h, whereas the peak concentration of PYOS (C_{\max} , 2524 ± 265 ng/mL) was observed
312 at 1.7 ± 0.6 hours. Furthermore, the AUC of PYOS was also larger than that of pyrene ($12677 \pm$
313 799 and 3375 ± 84 ng*h/mL, respectively). The $t_{1/2}$ of pyrene was longer than that of its
314 metabolite, PYOS (91.2 ± 68.2 and 3.6 ± 0.9 h, respectively). However, MRT (4.2 ± 0.8 h) and
315 CL/F (0.2 ± 0.01 L/h) of PYOS were shorter than those of the parent compound, pyrene (MRT,
316 134.5 ± 94.4 h; CL/F, 0.3 ± 0.1 L/h).

317

318 *3.4. Comparison of pyrene metabolizing ability among bird species and rats*

319 The enzyme kinetics activities were examined by *in vitro* study to observe sulfation (in
320 cytosol) and glucuronidation (in microsome) activities in chickens, quails, and rats using PYOH
321 as a substrate. The results for sulfotransferase activity are presented in Fig. 4A and Table 3. The
322 hepatic intrinsic clearance (V_{\max}/K_m), the maximum reaction rate (V_{\max}), and the Michaelis
323 constant (K_m) were examined to estimate conjugated enzyme affinities. The sulfotransferase
324 activity of PYOH was detected in both chicken and quail cytosols. Interestingly, interspecies
325 differences between rats and bird species were found, with V_{\max} and K_m of rat cytosol being
326 significantly greater than those of chicken and quail cytosols. Although, the V_{\max}/K_m in quail
327 cytosol was greater than those in chicken and rat cytosols (830 ± 352 , 608 ± 472 , and 429 ± 51.1
328 $\mu\text{L}/\text{min}/\text{mg}$ protein, respectively), the differences among species were not significant.
329 Furthermore, UGT activity of PYOH is also shown in Fig. 4B and Table 4. The V_{\max} of rat and
330 quail microsomes were similar and were significantly higher than that of chicken microsomes.
331 Surprisingly, the K_m of quail microsomes was significantly faster than those of rats and chicken.

332 Moreover, the V_{\max}/K_m of chicken and quail microsomes were significantly lower than that of rat
333 microsome (21.2 ± 1.39 , 33.1 ± 7.76 , and 80.8 ± 25.6 $\mu\text{L}/\text{min}/\text{mg}$ protein, respectively).

334 4. Discussion

335

336 Glucuronide and sulfate conjugated metabolites of pyrene were identified and detected in the
337 plasma and their excreta, including bile, of birds. The main conjugated metabolites were PYOG
338 and PYOS, which were detected at levels similar to those in various mammals, fish, reptiles,
339 amphibians, and marine snails (Beach et al., 2010; Ikenaka et al., 2013; Oroszlany et al., 2013;
340 Saengtienchai et al., 2014; Ueda et al., 2011). Interestingly, PYDOGG and PYDOGS were
341 detected in excreta and bile of birds, but not in urine or feces of rats. Although there have been
342 reports of glucuronide and sulfate conjugates of 1,6-PYDOH and 1,8-PYDOH, and *trans*-4,5-
343 PYDOH in urine of rats, rabbits, fish, reptiles, amphibians, and marine snails treated with pyrene
344 (Beach et al., 2010; Boyland et al., 1964; Ikenaka et al., 2013; Oroszlany et al., 2013;
345 Saengtienchai et al., 2014; Ueda et al., 2011), PYDOGG and PYDOGS were first reported in
346 birds. It has been reported that genetic polymorphism of conjugated enzymes, such as UGT1A
347 isoforms, differed among birds and mammals (Kakehi et al., 2015; Hong et al., 2007). The
348 present study showed interspecies differences in pyrene metabolites.

349 Furthermore, the portion of pyrene conjugated metabolites in plasma, excreta, and bile
350 showed interspecies differences between birds and rats. In plasma, sulfate conjugated
351 metabolites were mainly detected in both birds and rats. However, more than 90% of sulfate
352 conjugated metabolites observed in birds was the sulfate conjugate for PYOH, whereas the
353 around 90% of the metabolites observed from rats were for PYDOH. These results seemed to
354 parallel those of the *in vitro* study indicating that V_{\max}/K_m of PYOH sulfation in hepatic cytosol
355 of birds was higher than that of rats.

356 In urine and feces, conjugated metabolites of pyrene had various forms and unique
357 characteristics among species, including birds. Glucuronide conjugated metabolites were mainly
358 detected in the excreta of birds, whereas sulfate conjugated metabolites and PYOH were mostly
359 excreted into urine and feces of rats, respectively. In contrast, the V_{\max}/K_m of PYOH
360 glucuronidation in hepatic microsomes of birds was lower than that of rats *in vitro*. This
361 discrepancy may be explained by the potentially divergent biotransformation in birds and rats
362 due to differences in the pattern of xenobiotic elimination. With regard to xenobiotic
363 metabolism, the metabolizing organs above the liver, such as the kidney, lung, and intestines,

364 also showed phase II conjugation reactions with rapid passage of xenobiotics and/or their
365 metabolites from the circulation into the tissues (Saengtienchai et al., 2015). Moreover, the
366 ability of the transporters expressed in various tissues, such as multidrug resistance protein 2 and
367 breast cancer resistance protein, may be other factors that could drive excretion of sulfate and
368 glucuronide conjugate into urine and feces (Fink-Gremmels, 2010). On the other hand,
369 differences in biliary excretion could also explain the observed species-related differences in
370 xenobiotic metabolism, and the molecular weight has been suggested as an important factor in
371 biliary excretion (Abou-El-Makaren et al., 1967; Hirom et al., 1972). High molecular weight
372 compounds (in the range >355–550) could cause an apparent increase in excretion through
373 biliary excretion in rats, dogs, guinea pigs, rabbits, hen, and turkeys (Abou-El-Makaren et al.,
374 1967; Hirom et al., 1972; Smith et al., 1995; Smith et al., 2000). Meanwhile, the conjugated
375 metabolites with higher molecular weights were present in the bile of birds. However, the
376 percentage of conjugated pyrene metabolites was altered in the excreta associated with
377 biodegradation of pyrene conjugated metabolites by normal flora in the intestine (Kanaly and
378 Harayama, 2000; Saengtienchai et al., 2015). Conjugated pyrene metabolites present in urine and
379 feces of rats were markedly different from those in birds. Furthermore, Watanabe et al. (2010,
380 2015) previously reported a large interspecies difference in CYP-mediated metabolism in phase I
381 oxidation following treatment with warfarin. This may be because birds have unique xenobiotic
382 metabolism occurring in both phase I oxidation and phase II conjugation.

383 The pharmacokinetics findings provided an understanding of the influence of a single pyrene
384 exposure in chickens on absorption, metabolism, and elimination. In addition, the $t_{1/2}$ of pyrene in
385 chickens was longer than that of its metabolite, PYOS. The results of the present study indicated
386 a longer $t_{1/2}$ of pyrene than that reported in rats (4.9 h) (Bouchard et al., 1998; Withey et al.,
387 1991). This may have been because pyrene showed persistence in plasma with prolongation of
388 MRT and CL/F. Therefore, pyrene could be accumulated in various tissues and its metabolism
389 may also occur in these tissues (Saengtienchai et al., 2015). Meanwhile, PYOS was produced
390 rapidly and was eliminated with less residue in the chicken body. Moreover, Watanabe et al.
391 (2015) reported that the $t_{1/2}$ of warfarin in chickens was longer than in mammalian species. The
392 results of the present study indicated that xenobiotics seemed to accumulate in bird tissues
393 following a single exposure, which could be sufficient to cause toxicity.

394 5. Conclusions

395

396 In the present study, interspecies differences in xenobiotic metabolism were clarified among
397 bird and mammalian species. Pyrene was readily metabolized with conjugated enzymes from
398 birds upon single exposure, although pyrene itself had a longer elimination half-life in birds than
399 in rats. Moreover, conjugated metabolites, such as PYDOGG and PYDOGS, were first reported
400 in the present study. Further studies are required regarding the bioaccumulation of xenobiotics.
401 This may be related to the decreases in bird populations and may affect the ecosystem in the near
402 future.

403

404 Acknowledgments

405

406 This work was supported by Grants-in-Aid for Scientific Research from the Ministry of
407 Education, Culture, Sports, Science and Technology of Japan awarded to M. Ishizuka (No.
408 16H0177906), Y. Ikenaka (15H0282505, 17K2003807), S. Nakayama (16K16197), and the
409 foundations of the JSPS Core to Core Program (AA Science Platforms) and the Bilateral Joint
410 Research Project (PG36150002 and PG36150003). We also acknowledge the financial support of
411 Mitsui & Co., Ltd. Environment Fund, the Sumitomo Foundation for Environmental Research
412 Projects, the Soroptimist Japan Foundation, the Nakajima Foundation, and the Inui Memorial
413 Trust for Research on Animal Science. The authors are grateful to thank Mr. Takahiro Ichise, Ms.
414 Mio Yagihashi, and Ms. Nagisa Hirano for their technical support and input throughout this
415 research.

416

417 **References**

418

419 Abou-El-Makaren, M.M., Millburn, P., Smith, R.L., Williams, R.T. 1967. Biliary excretion of
420 foreign compounds: species differences in biliary excretion. *Biochem. J.* 105, 1289–1293.

421 Albers, P.H., Hoffman, D.J., Rattner, B.A., Burton, G.A., Cairns, J. 2003. Petroleum and
422 individual polycyclic aromatic hydrocarbons, *Handbook of Ecotoxicology*, second edition,
423 Chapter 14, pp341–371, Lewis Publishers, Boca Raton, FL.

424 Albert, C.A., Wilson, L.K., Mineau, P., Trudeau, S., Elliott, J.E. 2010. Anticoagulant
425 rodenticides in three owl species from Western Canada, 1983–2003. *Arch Environ. Contam.*
426 *Toxicol.* 58, 451–459.

427 Armstrong, B.G., Hutchinson, E., Unwin, J., Fletcher, T. 2004. Lung Cancer Risk after Exposure
428 to Polycyclic Aromatic Hydrocarbons: A Review and Meta-Analysis. *Environ. Health*
429 *Perspect.* 112, 970–978.

430 Beach, D.G., Quilliam, M.A., Rouleau, C., Croll, R.P., Hellou, J. 2010. Bioaccumulation and
431 biotransformation of pyrene and 1-hydroxypyrene by the marine whelk *Buccinum undatum*.
432 *Environ. Toxicol. Chem.* 29, 779–788.

433 Boocock, D.J., Patel, K.R., Faust, G.E.S., Normolle, D.P., Marczyklo, T.H., Crowell, J.A.,
434 Brenner, D.E., Booth, T.D., Gescher, A., Steward, W.P. 2007. Quantitation of trans-
435 resveratrol and detection of its metabolites in human plasma and urine by high performance
436 liquid chromatography. *J. Chromatogr. B* 848, 182–187.

437 Bouchard, M., Krishnan, K., Viau, C. 1998. Kinetics of tissue distribution and elimination of
438 pyrene and 1-hydroxypyrene following intravenous administration of [¹⁴C]pyrene in rats.
439 *Toxicol. Sci.* 46, 11–20.

440 Bowerman, W.W., Giesy, J.P., Best, D.A., Krame, V.J. 1995. A Review of Factors Affecting
441 Productivity of Bald Eagles in the Great Lakes Region: Implications for Recovery. *Environ.*
442 *Health Perspect.* 103, 51–59.

- 443 Boyland, E., Sims, P. 1964. Metabolism of polycyclic compounds. 23. The metabolism of pyrene
444 in rats and rabbits. *Biochem. J.* 90, 391–398.
- 445 Erickson, W., Urban, D. 2004. Potential risks of nine rodenticides to birds and nontarget
446 mammals: a comparative approach. Environmental Protection Agency Office of Prevention,
447 Pesticides and Toxic Substances. Washington, D.C., USA.
- 448 Elliott, J.E., Norstrom, R.J., Keith, J.A. 1988. Organochlorines and eggshell thinning in northern
449 gannets (*Sula bassanus*) from Eastern Canada, 1968–1984. *Environ. Pollut.* 52, 81–102.
- 450 Fink-Gremmels, J. 2009. Defense mechanisms against toxic phytochemicals in the diet of
451 domestic animals. *Mol. Nutr. Food Res.* 54, 249–258.
- 452 Hirom, P.C., Millburn, P., Smith, R.L., Williams, R.T. 1972. Species variations in the threshold
453 molecular-weight factor for the biliary excretion of organic anions. *Biochem. J.* 129, 1071–
454 1077.
- 455 Hong, A., Huo, D., Kim, H., Niu, Q., Fackenthal, D., Cummings, S., John, E., West, D.,
456 Whittemore, A., Das, S., Olopade OI. 2007. UDP-Glucuronosyltransferase 1A1 Gene
457 Polymorphisms and Total Bilirubin Levels in an Ethnically Diverse Cohort of Women.
458 *Drug. Metab. Dispos.* 35, 1254–1261.
- 459 Ikenaka, Y., Ishizaka, M., Eun, H., Miyabara, Y. 2007. Glucose–sulfate conjugates as a new
460 phase II metabolite formed by aquatic crustaceans. *Biochem. Biophys. Res. Commun.* 360,
461 490–495.
- 462 Ikenaka, Y., Oguri, M., Saengtienchai, A., Nakayama, S.M.M., Ijiri, S., Ishizuka, M. 2013.
463 Characterization of phase-II conjugation reaction of polycyclic aromatic hydrocarbons in
464 fish species: unique pyrene metabolism and species specificity observed in fish species.
465 *Environ. Toxicol. Pharmacol.* 36, 567–578.
- 466 Jiang, Y.F., Wang, X.T., Wu, M.H., Sheng, G.Y., Fu, J.M. 2011. Contamination, source
467 identification, and risk assessment of polycyclic aromatic hydrocarbons in agricultural soil
468 of Shanghai, China. *Environ. Monit. Assess.* 183, 139–150.
- 469 Kakehi, M., Ikenaka, Y., Nakayama, M.M.S., Kawai, Y., Watanabe, K., Mizukawa, H.,
470 Nomiyama, K., Tanabe, S., Ishizuka, M. 2015. Uridine Diphosphate-

- 471 Glucuronosyltransferase (UGT) Xenobiotic Metabolizing Activity and Genetic Evolution in
472 Pinniped Species. *Toxico. Sci.* 147, 360–369.
- 473 Kanaly, R.A., Harayama, S. 2000. Biodegradation of high-molecular-weight polycyclic aromatic
474 hydrocarbons by bacteria. *J. Bacteriol.* 182, 2059–2067.
- 475 Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurement with the
476 Folin phenol reagent. *J. Biol. Chem.* 193, 265–275.
- 477 Norstrom, R., Hebert, C.E. 2006. Comprehensive re-analysis of archived herring gull eggs
478 reconstructs historical temporal trends in chlorinated hydrocarbon contamination in Lake
479 Ontario and Green Bay, Lake Michigan, 1971–1982. *J. Environ. Monit.* 8, 835–847.
- 480 Omura, T., Sato, R. 1964. The carbon monoxide-binding pigment of liver microsomes. *J. Biol.*
481 *Chem.* 239, 2370–2378.
- 482 Oroszlany, B., Ikenaka, Y., Saengtienchai, A., Oguri, M., Nakayama, S.M.M., Ishizuka, M.
483 2013. Metabolism of pyrene, a polycyclic aromatic hydrocarbon in freshwater turtles. *Jpn.*
484 *J. Vet. Res.* 61, S62–S63.
- 485 Prakash, V., Green, R.E., Pain, D.J., Ranade, S.P., Saravanan, S., Prakash, N., Venkitachalam,
486 R., Cuthbert, R., Rahmani, A.R., Cunningham, A.A. 2007. Recent Changes in Populations
487 of Resident Gyps Vultures in India. *J. Bombay Nat. His. Soc.* 104, 129–135.
- 488 Saengtienchai A, Ikenaka Y, Nakayama, S.M.M., Mizukawa, H., Kakehi, M., Bortey-Sam, N.,
489 Darwish, W.S., Tsubota, T., Terasaki, M., Poapolathep, A., Ishizuka, M. 2014.
490 Identification of interspecific differences in phase II reactions: determination of metabolites
491 in the urine of 16 mammalian species exposed to environmental pyrene. *Environ. Toxicol.*
492 *Chem.* 33, 2062–2069.
- 493 Saengtienchai, A., Ikenaka, Y., Darwish, W.S., Nakayama, S.M.M., Mizukawa, H., Ishizuka, M.
494 2015. Characterization and tissue distribution of conjugated metabolites of pyrene in the rat.
495 *J. Vet. Med. Sci.* 77, 1261–1267.
- 496 Saengtienchai, A., Ikenaka, Y., Bortey-Sam, N., Jermnark, U., Mizukawa, H., Kawai, Y.K.,
497 Nakayama, S.M.M., Ishizuka, M. 2016. The African hedgehog (*Atelerix albiventris*): Low

- 498 phase I and phase II metabolism activities. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*
499 190, 38–47.
- 500 Smith, D.J., Giddings, J.M., Feil, V.J., Paulson, G.D. 1995. Identification of ractopamine
501 hydrochloride metabolites excreted in rat bile. *Xenobiotica* 25, 511–520.
- 502 Smith, D.J., Feil, V.J., Paulson, G.D. 2000. Identification of turkey biliary metabolites of
503 ractopamine hydrochloride and the metabolism and distribution of
504 synthetic[¹⁴C]ractopamine glucuronides in the turkey. *Xenobiotica* 30, 427–440.
- 505 Ueda, H., Ikenaka, Y., Nakayama, S.M.M., Tanaka-Ueno, T., Ishizuka, M. 2011. Phase-II
506 conjugation ability for PAH metabolism in amphibians: characteristics and inter-species
507 differences. *Aquat. Toxicol.* 105, 337–343.
- 508 Watanabe, K.P., Saengtienchai, A., Tanaka, K.D., Ikenaka, Y., Ishizuka, M. 2010. Comparison
509 of warfarin sensitivity between rat and bird species. *Comp. Biochem. Physiol. C Toxicol.*
510 *Pharmacol.* 152, 114–119.
- 511 Watanabe, K.P., Kawata, M., Ikenaka, Y., Nakayama, S.M.M., Ishii, C., Darwish, W.S.,
512 Saengtienchai, A., Mizukawa, H., Ishizuka, M. 2015. Cytochrome P450-mediated metabolic
513 ability is not a critical determinant of warfarin sensitivity in avian species: in vitro assays in
514 several birds and in vivo assays in chicken. *Environ. Toxicol. Chem.* 34, 2328–2334.
- 515 Withey, J.R., Law, F.C., Endrenyi, L. 1991. Pharmacokinetics and bioavailability of pyrene in
516 the rat. *J. Toxicol. Environ. Health* 32, 429–447.
- 517
- 518

519 Figure Legends

520

521 Fig. 1. Chromatograms of pyrene metabolites in chicken bile after oral administration and
522 analysis by HPLC/FD. Peak-a, b, c, d, e, and f represent the pyrene metabolites detected, and
523 included PYOH (peak-g) and pyrene (peak-h). Peak-a was a PYDOGS, peak-b was a PYDOGG,
524 peak-c was a PYDOSS, peak-d was a PYDOS, peak-e was a PYOG, and peak-f was a PYOS.
525 Excitation (Ex) and emission (Em) wavelengths for FD were 343 and 385 nm, respectively.

526

527 Fig. 2. Percentages of each pyrene metabolite in plasma, excreta, bile, and urine contained in the
528 ureter in chickens ($n=3$); plasma and excreta in quails ($n=3$); plasma, urine, and feces in rats (n
529 $=3$). These percentages were obtained from the area of each metabolite peak detected by
530 HPLC/FD. Each pyrene metabolite structure is indicated in color.

531

532 Fig. 3. Distribution profiles between pyrene and PYOS and time (h) in chicken plasma. Each
533 point represents the average peak area \pm SD, $n=3$.

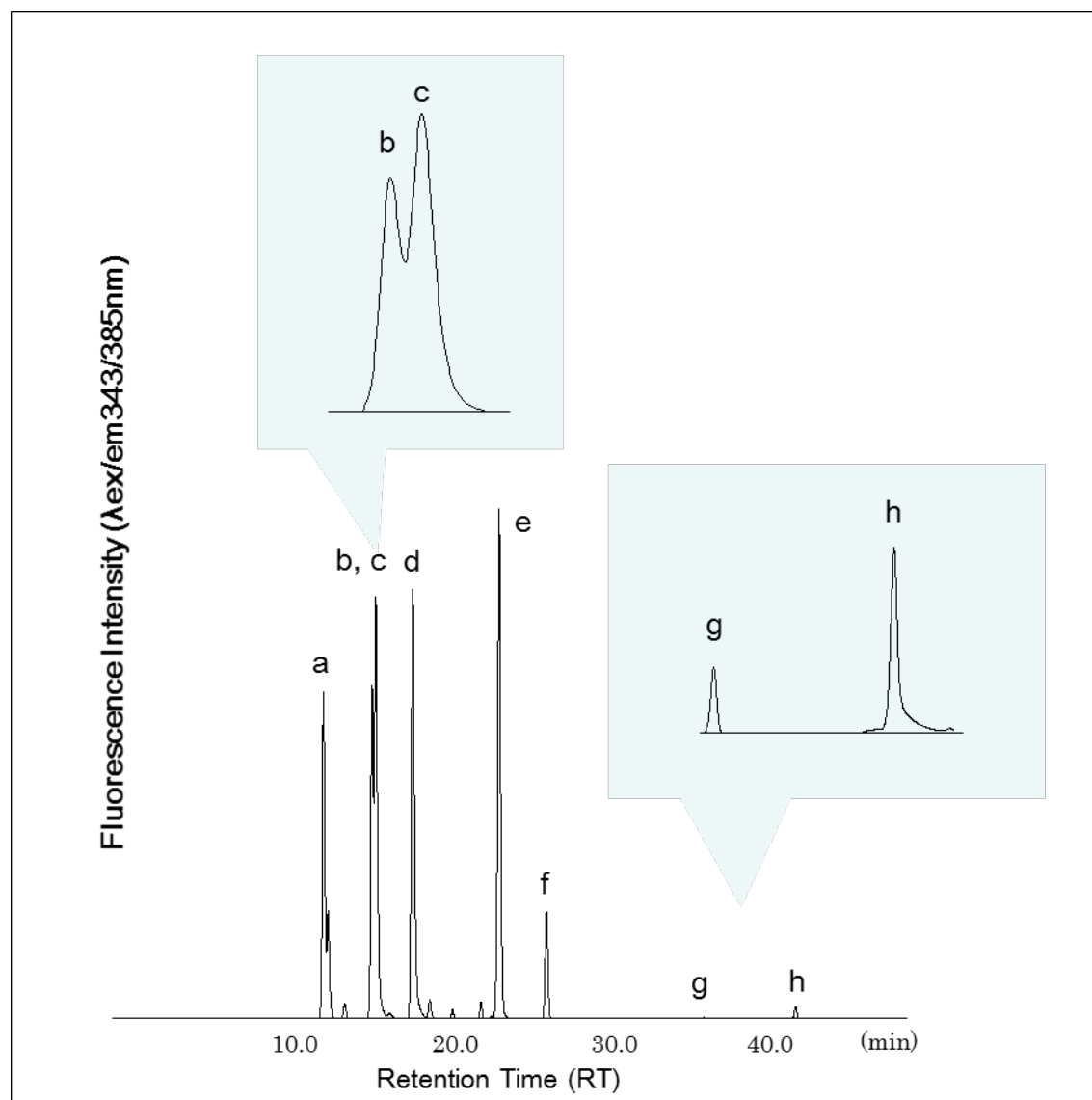
534

535 Fig. 4. Enzyme kinetics of SULT activity (4A) and UGT activity (4B) in rats and bird species.
536 The Michaelis–Menten equation was fitted and compared between rats, chickens, and quails.
537 Plots show the average \pm SD, $n=3$, each species.

538

539

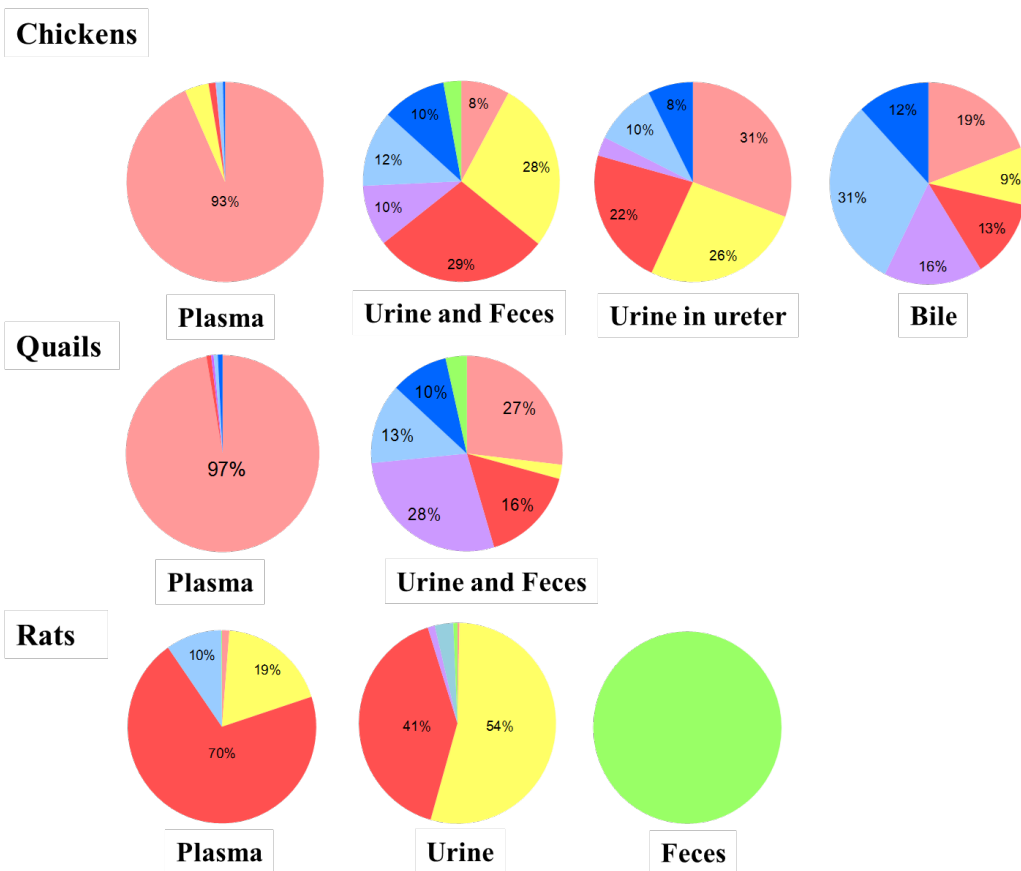
540 Fig. 1.



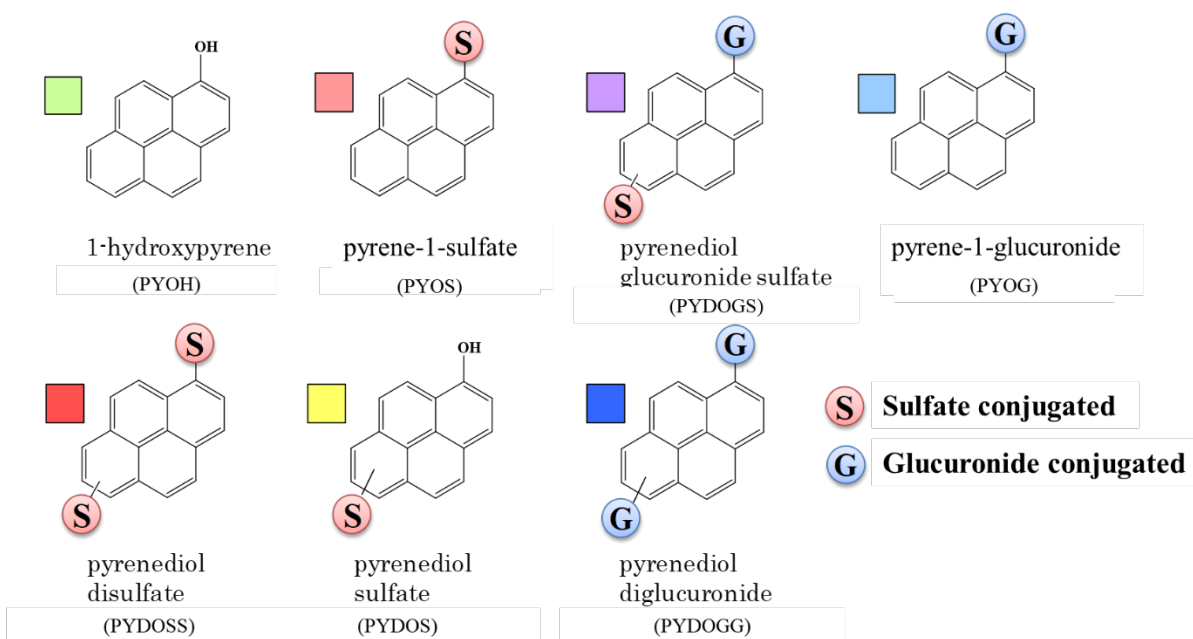
541

542

543 Fig. 2.

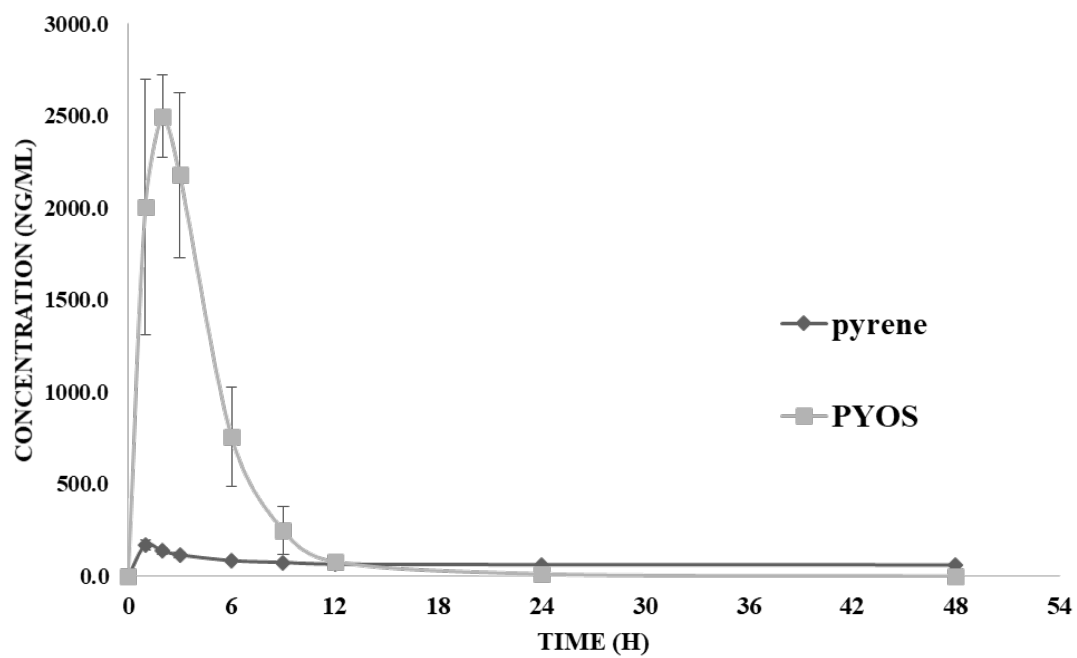


544



545

546 Fig. 3.



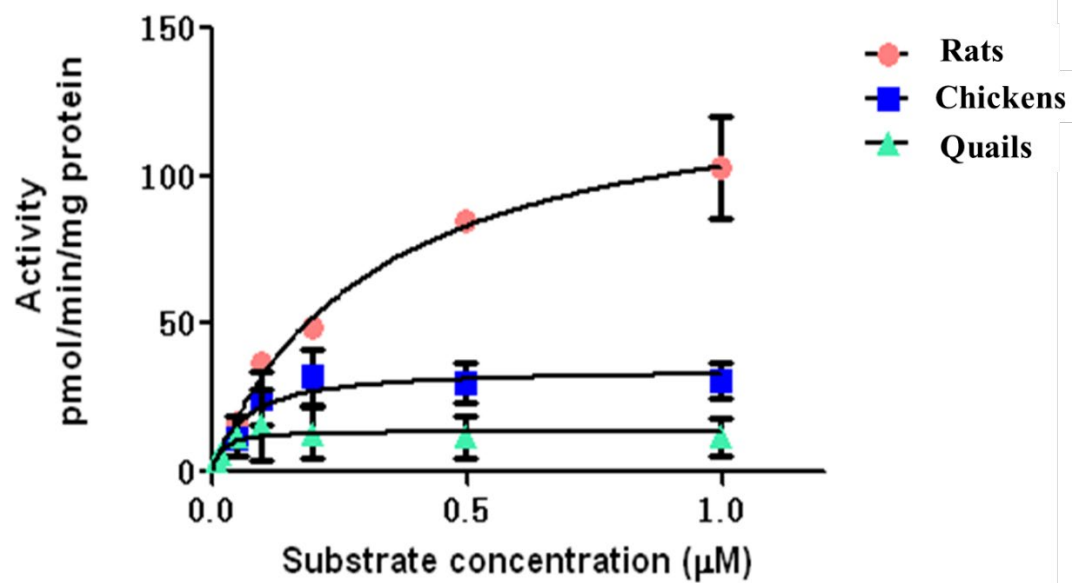
547

548

549

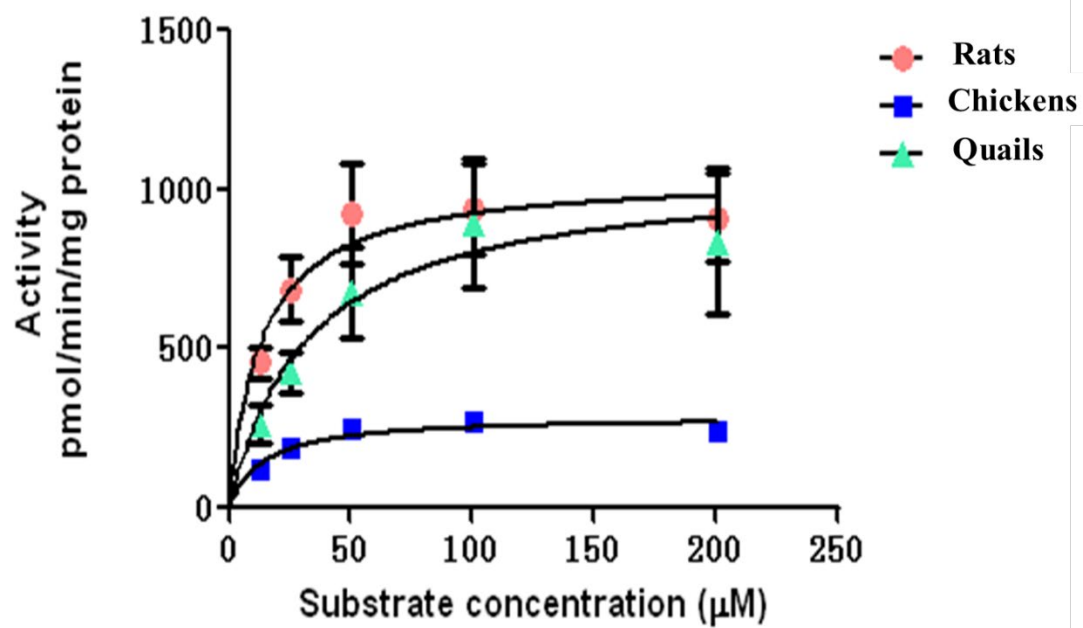
550 Fig. 4.

551 A.



552

553 B.



554

555

556 Table 1. Pyrene and its metabolites under MS, MS/MS, and deconjugation conditions in chicken
 557 bile

558

	RT (min)	MS (<i>m/z</i>)	MS ² (<i>m/z</i>)	Deconjugation reaction		Metabolites
				Sulfatase	β -glucuronidase	
Peak-a	11.6	489	409 313 233	++	++	PYDOGS
Peak-b	14.8	585	409	-	++	PYDOGG
Peak-c	15.0	393	313 233	++	-	PYDOSS
Peak-d	17.4	313	233	++	-	PYDOS
Peak-e	22.9	393	217	-	+	PYOG
Peak-f	25.9	297	217	++	-	PYOS
PYOH	36.0		217	-	-	
Pyrene	41.8			-	-	

559

560 -: no change

561 +: peak reduction 10%

562 ++: peak disappearance

563

564 Table 2. Pharmacokinetic parameters of pyrene and PYOS after oral administration of pyrene (4
565 mg/kg) in chickens (mean±SD), $n=3$

566

Parameters	Pyrene	PYOS
AUC (ng*h/mL)	3375±84	12677±799
C_{max} (ng/mL)	169±28	2524±265
T_{max} (h)	–	1.7±0.6
t_{1/2} (h)	91.2±68.2	3.6±0.9
MRT (h)	134.5±94.4	4.2±0.8
CL/F (L/h)	0.3±0.1	0.2±0.01

567

568 AUC, area under the curve; C_{max}, maximum plasma concentration; T_{max}, time to maximum
569 plasma concentration; t_{1/2}, elimination half-life; MRT, mean residence time; CL/F, total clearance
570 of the drug from plasma after oral administration

571

572 Table 3. Kinetic analysis of hepatic cytosol sulfotransferase activity toward PYOH in rats,
 573 chickens, and quails

574

	V_{\max} (pmol/min/mg protein)	K_m (μ M)	V_{\max}/K_m (μ L/min/mg protein)
Rats	136 \pm 30.8^a	0.32 \pm 0.11^a	429 \pm 51.1^a
Chickens	34.9 \pm 8.04^b	0.057 \pm 0.03^b	608 \pm 472^a
Quails	13.9 \pm 8.89^b	0.017 \pm 0.004^b	830 \pm 352^a

575

576 Mean \pm SD, $n=3$ 577 ^{a,b}Tukey's HSD, $p < 0.05$

578 V_{\max} = maximum velocity (pmol/min/mg protein); K_m = Michaelis constant (μ M); V_{\max}/K_m (μ L
 579 /min/mg protein)

580

581 Table 4. Kinetic analysis of hepatic microsomes UDP-glucuronosyltransferase activity toward
 582 PYOH in rats, chickens, and quails

583

	V_{\max} (pmol/min/mg protein)	K_m (μ M)	V_{\max}/K_m (μ L/min/mg protein)
Rats	1,044 \pm 175^a	13.1 \pm 5.57^b	80.8 \pm 25.6^a
Chickens	287 \pm 11.6^b	13.5 \pm 0.92^b	21.2 \pm 1.39^b
Quails	1,057 \pm 308^a	31.9 \pm 9.46^a	33.1 \pm 7.76^b

584

585 Mean \pm SD, $n=3$ 586 ^{a,b}Tukey's HSD, $p < 0.05$

587 V_{\max} = maximum velocity (pmol/min/mg protein); K_m = Michaelis constant (μ M); V_{\max}/K_m (μ L
 588 /min/mg protein)