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# Oxidation of 3-aminobenzanthrone, a human metabolite of carcinogenic environmental pollutant 3-nitrobenzanthrone, by cytochromes P450 – similarity between human and rat enzymes

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Running headline: 3-Aminobenzanthrone oxidation by cytochromes P450

**OBJECTIVES:** 3-Aminobenzanthrone (3-ABA) is the main human metabolite of carcinogenic environmental pollutant 3-nitrobenzanthrone (3-NBA). Understanding which cytochrome P450 (CYP) enzymes are involved in metabolism of this toxicant is important in the assessment of individual susceptibility. Characterization of 3-ABA metabolites formed by rat hepatic microsomes containing cytochromes P450 (CYPs)

and identification of the major rat and human CYPs participating in this process are aims of this study.

**METHODS:** HPLC with UV detection was employed for the separation and characterization of 3-ABA metabolites. Inducers and inhibitors of CYPs and rat and human recombinant CYPs were used to characterize the enzymes participating in 3-ABA oxidation.

**RESULTS:** Selective CYP inhibitors and hepatic microsomes of rats pre-treated with specific CYP inducers were used to characterize rat liver CYPs metabolizing 3-ABA (measured as consumption of 3-ABA). Kinetics of these reactions catalyzed by rat hepatic microsomes was also evaluated. Based on these studies, we attribute most of 3-ABA metabolism in rat liver to CYP1A and 3A. Among recombinant rat and human CYP enzymes tested in this study, rat CYP3A2 and human CYP3A4/5, followed by CYP1A1 of both organisms were the most effective enzymes converting 3-ABA. Rat hepatic CYP enzymes oxidize 3-ABA up to three metabolites. Two of them were identified to be the products formed by oxidation of 3-ABA on its amino group back to the parent compound from which 3-ABA is generated in organisms, 3-NBA. Namely, *N*-hydroxylation metabolite, *N*-hydroxy-3-ABA and 3-NBA were identified to be these 3-ABA oxidation products. These metabolites are formed by CYPs of a 1A subfamily. Another 3-ABA metabolite, whose structure remains to be characterized, is generated not only by CYP1A but also by other CYP enzymes, predominantly by CYPs of a 3A subfamily.

**CONCLUSIONS:** The results found in this study, the first report on the metabolism of 3-ABA by human and rat CYPs, clearly demonstrate that CYPs of 3A and 1A subfamilies are the major enzymes metabolizing 3-ABA.

50 **KEY WORDS**

51 3-aminobenzanthrone; 3-nitrobenzanthrone, cytochrome P450; metabolism.

52

53 **ABBREVIATIONS & UNITS**

54 3-ABA – 3-aminobenzanthrone

55 α-NF - α-naphthoflavone

56 β-NF - β-naphthoflavone

57 cDNA - complementary DNA

58 CYP - cytochrome P450

59 DDTC - diethyldithiocarbamic acid

60 HPLC - high performance liquid chromatography

61 $K_m$ - Michaelis constant

62 MS - microsomes

63 *N*-hydroxy-3-ABA - *N*-hydroxy-3- aminobenzanthrone

64 NADPH - nicotinamidadeninedinucleotide phosphate (reduced)

65 3-NBA – 3-nitrobenzatntrone

66 PB – phenobarbital

67 RP – reverse phase

68 r.t. – retention time

69 UV – ultraviolet

70 VIS - visible

71 $V_{max}$ - maximum reaction rate

72

73 **INTRODUCTION**

74 The nitroaromatic compound 3-nitrobenzanthrone (3-nitro-7H-
75 benz[de]anthracen-7-one, 3-NBA, Fig. 1) is one of the most potent mutagens and a
76 suspected human carcinogen that is found in diesel exhaust and ambient air pollution
77 (Arlt, 2005, Hansen et al., 2007). We found that 3-NBA is activated to *N*-hydroxy-3-
78 aminobenzanthrone (N-hydroxy-3-ABA) by cytosolic and microsomal reductases by
79 simple nitroreduction (Arlt 2005, Arlt et al., 2002, 2003a,b,c, 2005, Stiborova et al.,
80 2006a, 2008, 2009, Svobodova et al. 2007) (Figure 1).

81 Recently 3-NBA has received much attention due to its extremely high
82 mutagenic potency in the Ames Salmonella assay (Enya et al., 1997; Seidel et al.,
83 2002; Arlt, 2005). 3-NBA is carcinogenic in rats, causing lung tumours after
84 intratracheal instillation, and it is also a suspected human carcinogen (Seidel et al.,
85 2002; Arlt, 2005; Nagy et al., 2005).

86 The uptake of 3-NBA in humans has been demonstrated by the detection of its
87 metabolite 3-aminobenzanthrone (3-ABA, Figure 1) in urine samples of salt mine
88 workers occupationally exposed to diesel emissions (Seidel et al., 2002). 3-ABA was
89 also the main metabolite of 3-NBA formed in human fetal bronchial cells and rat lung
90 alveolar type II cells (Borlak et al., 2000).

91 3-ABA was also evaluated to be suitable for coloration of microporous
92 polyethylene films, which are widely used for practical purposes such as separation
93 of liquid mixtures, in particular, as separation membranes in chemical batteries
94 (Grabchev et al., 2002), or an advantageous fluorescent phospholipid membrane
95 label in the form of its *N*-palmitoyl derivative (Sykora et al., 2002). This suggests
96 industrial and/or laboratory utilization of this 3-NBA metabolite, leading to a putative
97 exposure of people. This is a matter of concern, because we have demonstrated the

98 genotoxicity of both 3-NBA and 3-ABA by the detection of specific DNA adducts
99 formed *in vitro* and *in vivo* (Arlt et al., 2001; 2002; 2003a; b; c; 2004a; c; 2005;
100 2006b; Bieler et al., 1999; 2005; 2007; Stiborova et al., 2006a; 2008, 2009). Previous
101 work indicated that *N*-hydroxy-3-ABA appears to be the critical intermediate in 3-
102 NBA/ABA-derived DNA adduct formation (Figure 1), which can be further activated
103 by *N,O*-acetyltransferases (NATs) and sulfotransferases (SULTs) (Arlt et al., 2002,
104 2003a, b, 2005). The predominant DNA adducts formed from 3-NBA and 3-ABA are
105 2-(2'-deoxyguanosin-$N^2$-yl)-3-aminobenzanthrone (dG-$N^2$-ABA) and *N*-(2'-
106 deoxyguanosin-8-yl)-3-aminobenzanthrone (dG-C8-*N*-ABA) and these are most
107 probably responsible for the induction of GC to TA transversion mutations induced
108 by these toxicants (Arlt et al., 2004a; Arlt et al., 2006b; Bieler et al., 2007).
109 Understanding which enzymes are involved in the metabolism (activation
110 and/or detoxication) of 3-ABA is important in the assessment of susceptibility to this
111 3-NBA metabolite. Recently, we have found that cytochromes P450 (CYP) 1A1 and
112 1A2 are essential for 3-ABA oxidative activation in human and rat liver, lung and
113 kidney to reactive species, *N*-hydroxy-3-ABA, forming the same DNA adducts that
114 are formed *in vitro* and *in vivo* in rodents by 3-ABA or 3-NBA (Arlt et al. 2003a, b, c,
115 2004b, 2005, 2006a, Stiborova et al. 2006a; 2008, 2009) (Figure 1).
116 In contrast to the enzymes activating 3-ABA to species binding to DNA, those
117 participating in 3-ABA oxidation to other potential metabolites have not been
118 extensively studied so far. Therefore, here we investigated the oxidative metabolism
119 of 3-ABA *in vitro*, in order to characterize the 3-ABA metabolites and to identify CYPs
120 responsible for their formation. Hepatic microsomal systems and recombinant CYP
121 enzymes of rat, the organism found previously to be a suitable model mimicking
122 activation metabolism of 3-ABA in human (Arlt et al., 2004b) were used for such a

123 study. In addition, rat and human recombinant CYP enzymes were utilized to
124 characterize their participation in 3-ABA oxidation.
125

126 **MATERIAL AND METHODS**

127 *Synthesis of 3-ABA and N-hydroxy-3-ABA.* 3-ABA and N-hydroxy-3-ABA were
128 synthesized as described by Arlt et al. (2003a) and their authenticity was confirmed
129 by UV spectroscopy, electrospray mass spectra and high field proton NMR
130 spectroscopy.

131 *Chemicals and enzymes.* Microsomes from rat livers were isolated and characterized
132 for CYP activities as described (Arlt et al., 2004b, Stiborova et al., 2006a,b).
133 Supersomes™, microsomes isolated from insect cells transfected with Baculovirus
134 constructs containing cDNA of one of the following rat CYPs: CYP1A1, 1A2, 2A1,
135 2A2, 2B1, 2C6, 2C11, 2C12, 2C13, 2D1, 2D2, 2E1, 3A1, 3A2 with cytochrome $b_5$ and
136 expressing NADPH:CYP reductase and one of the following human CYPs: CYP1A1,
137 1A2, 2A6, 2B6, 2C8 (with and without cytcohrome $b_5$), 2C19 (with and without
138 cytochrome $b_5$),, 2D6, 2E1, 3A4 and 3A5 (with and without cytcohrome $b_5$), and
139 expressing NADPH:CYP reductase were obtained from Gentest Corp. (USA).
140 NADPH, phenacetine, dimethylsulfoxide (DMSO) were obtained from Sigma
141 Chemical Co (St Louis, MO, USA), ethylacetate, methanol for high performance
142 liquid chromatography (HPLC) super gradient, methanol were obtained from
143 Lachema, (Brno, Czech Republic).

144 *Animal experiments and preparation of microsomes.* The study was conducted in
145 accordance with the Regulations for the Care and Use of Laboratory Animals
146 (311/1997, Ministry of Agriculture, Czech Republic), which complies with the
147 Declaration of Helsinki. Microsomes from livers of ten male untreated Wistar rats and

148 those from livers of ten male rats pre-treated with β-naphtoflavone (β-NF) and
149 phenobarbital (PB) were prepared by the procedure described previously (Arlt et al.,
150 2004b, Stiborova et al., 2002b, 2006b,. Krizkova et al., 2008, Sistkova et al., 2008).
151 Protein concentrations in the microsomal fractions were assessed using the
152 bicinchoninic acid protein assay with the bovine serum albumin as a standard
153 (Weichelman et al., 1988). The concentration of CYP was estimated according to
154 Omura and Sato (Omura and Sato, 1964) based on absorption of the complex of
155 reduced CYP with carbon monoxide. Untreated rat liver microsomes contained 0.6
156 nmol CYP/mg protein. Hepatic microsomes of rats treated with β-NF and PB
157 contained 1.3 and 1.5 nmol CYP/mg proteins, respectively.
158 *Incubations.* Unless stated otherwise, incubation mixtures used for studying 3-ABA
159 metabolism by rat hepatic microsomes were prepared as described previously by
160 Mizerovska et al. (2008). Briefly, incubation mixtures, containing final volume of 500
161 µl, consisted of 100 mM potassium phosphate buffer (pH 7.4), 10 mM NADPH, 0.5
162 mg of microsomal protein and 5 - 50 µM 3-ABA (dissolved in DMSO). The reaction
163 was initiated by adding 3-ABA. Incubations with rat microsomes were carried out at
164 37 °C for 5 minutes. Control incubations were carried out either without the
165 enzymatic system (microsomes) or without NADPH. Incubation mixtures used for
166 studying 3-ABA metabolism by rat and human recombinant CYP enzymes contained
167 final volume of 250 µl, consisting of 100 mM potassium phosphate buffer (pH 7.4), 10
168 mM NADPH, 1 µM CYP in Supersomes™ and 20 µM 3-ABA (dissolved in DMSO).
169 All incubations were carried out at 37 °C for 20 min. In the case of investigation of the
170 time dependence of 3-ABA oxidation, reaction mixtures were incubated at 37°C for 0
171 - 120 minutes. Control incubations were carried out either without the CYP enzymes
172 (Supersomes™) or without NADPH. Then, 2.5 or 5 µl of 1 mM phenacetine in

173 methanol was added as an internal standard, and 3-ABA and its metabolites were
174 extracted twice with ethyl acetate (2 x 1.5 ml). The extracts were evaporated to
175 dryness; residues dissolved in 30 µl of methanol and subjected to reverse-phase
176 (RP)-HPLC to evaluate the amounts of residual 3-ABA and its metabolites. The 3-
177 ABA metabolites were separated from 3-ABA by HPLC with UV detection and
178 characterized by mass spectrometry and co-chromatography with synthetic
179 standards. The following chemicals were used in the inhibition studies of the 3-ABA
180 oxidation by hepatic microsomes: α-naphthoflavone (α-NF), which inhibits CYP1A1
181 and 1A2; furafylline, which inhibits CYP1A2, diamantane, which inhibits CYP2B
182 (Stiborova et al., 2002a); sulphafenazole, which inhibits CYP2C;
183 diethyldithiocarbamic acid (DDTC), which inhibits CYP2E1 and ketoconazole, which
184 inhibits CYP3A (Rendic & DiCarlo, 1997). Inhibitors were dissolved in ethanol, except
185 of α-NF that was dissolved in a mixture of methanol:ethylacetate (3:2, v/v) and DDTC
186 that was dissolved in distilled in water, to yield final concentrations of 1-1000 µM in
187 the incubation mixtures.

188 *HPLC.* HPLC was performed with a reversed phase column (Nucleosil 100-5 $C_{18}$,
189 Macherey-Nagel, Duren, Germany, 25 cm x 4.6 mm, 5 mm) proceeded by a C-18
190 guard column, using isocratic elution conditions of 70% methanol in distilled water
191 with a flow rate of 0.6 ml/min. HPLC was carried out with a Dionex HPLC pump P580
192 with UV/VIS UVD 170S/340S spectrophotometer detector set at 254 nm, and peaks
193 were integrated with a CHROMELEON™ 6.01 integrator. 3-ABA, *N*-hydroxy-3-ABA,
194 and 3-NBA were eluted with retention times (r.t.) of 8.2, 6.5 and 25 minutes,
195 respectively (Figure 2)

196

197 **RESULTS**

198 When 3-ABA was incubated with rat hepatic microsomes in the presence of
199 NADPH up to three product peaks were separated by HPLC (see Figure 2 for hepatic
200 microsomes of rats treated with β-NF). Using co-chromatography with synthetic
201 standards, two of them were identified to be the products formed by oxidation of 3-
202 ABA on its amino group back to the parent compound from which 3-ABA is
203 generated in organisms, 3-NBA. Namely, *N*-hydroxylation metabolite, *N*-hydroxy-3-
204 ABA and 3-NBA were identified to be these 3-ABA oxidation products. This finding
205 indicates that 3-ABA might, in some cases, be oxidized through *N*-hydroxy-3-ABA
206 back to its oxidative counterpart, 3-NBA (Figure 1). A structure of another metabolite
207 eluted with the retention time (r.t.) of 18 min, M18 (Figure 2A) remains to be
208 characterized.

209 We have used hepatic microsomes of rats treated with CYP inducers (an inducer
210 of CYP1A1/2, β-NF and an inducer CYP2B1/2, PB), as well as inhibitors of individual
211 CYPs, to evaluate the role of rat hepatic CYPs in 3-ABA metabolism (Mizerovska et
212 al., 2008). Here, the same microsomal systems and hepatic microsomes of control
213 (untreated) rats were utilized to determine the kinetics of 3-ABA metabolism. If 3-ABA
214 was incubated with these microsomes more than 30 minutes, different patterns of 3-
215 ABA metabolic products were found in individual microsomes. Whereas hepatic
216 microsomes rich in CYP1A1/2 (β-NF-microsomes) generated the final oxidation
217 metabolite of 3-ABA, 3-NBA (Figure 1), this metabolite (3-NBA) was not formed by
218 the other microsomes tested in the study (Figure 3). The metabolite with unknown
219 structure, M18, was the only one formed from 3-ABA by these enzymatic systems
220 (Figure 3). It should be mentioned that in some cases the control incubations without
221 presence of microsomes contained traces of 3-ABA metabolites, 3-NBA and a
222 metabolite M18 (see Figure 3A showing this case), which might probably be formed

223 by autooxidation. *N*-hydroxy-3-ABA was another metabolite generated by
224 microsomes rich in CYP1A1/2 (Figure 2A), but this metabolite was not quantified in
225 this study, namely, because this reactive compound is easily decomposed and forms
226 nitrenium and/or carbenium ion (Figure 1), being scavenged by proteins (at least
227 partially) present in the incubation mixtures. When shorter incubation times were
228 used in the experiments, formation of all 3-ABA oxidation products detected in the
229 above experiments was low, being hardly to be quantified. Hence, because the
230 incubation times during them the conversion of 3-ABA is linear (that are shorter than
231 30 minutes, see below) are needed to study kinetics of 3-ABA metabolism by
232 microsomes, the consumption of 3-ABA, detected during such short incubation times,
233 has been used to evaluate initial velocities of the enzymatic reactions. Conversion of
234 3-ABA by all three hepatic microsomes (measured by consumption of 3-ABA) was
235 linear up to 5 minutes of incubation (data not shown). This time interval was,
236 therefore, used to evaluate kinetics of 3-ABA oxidation.

237 A classical Michaelis-Menten kinetics was found for 3-ABA metabolism by
238 hepatic microsomes of rats treated with β-NF and PB; double reciprocal plots of initial
239 velocities *versus* concentrations of 3-ABA were linear (Figure 4A,B). The values of
240 Michaelis constant ($K_m$) and maximum reaction velocity ($V_{max}$) determined from this
241 kinetics are shown in Table 1. The values of $K_m$ and $V_{max}$ for 3-ABA metabolism by
242 PB-microsomes were more than 1.2-fold higher than those by hepatic microsomes of
243 rats treated with β-NF.

244 In contrast to the kinetics found with rat hepatic β-NF- and PB-microsomes,
245 double reciprocal plots of kinetics of 3-ABA oxidation by hepatic microsomes of
246 control (untreated) rats were not linear (Figure 4C), giving two $K_m$ values for 3-ABA
247 (Table 1). These findings indicate that more than one of the CYP enzymes present in

248 these microsomes are responsible for 3-ABA metabolism. Because one of the $K_m$
249 values is close to the value of $K_m$ found with microsomes rich in CYP1A1/2 (β-NF-
250 microsomes), the enzyme of a CYP1A subfamily might be important for 3-ABA
251 conversion in hepatic microsomes of control rats. Indeed, inhibitors of CYPs of a 1A
252 subfamily (α-NF for CYP1A1/2 and furafylline for CYP1A2) are strong inhibitors of 3-
253 ABA metabolism (Table 2). Results of experiments utilizing CYP inhibitors also
254 suggest that CYP enzymes of a 3A subfamily might participate in 3-ABA metabolism
255 in rat hepatic microsomes; a selective CYP3A inhibitor, ketoconazole, was highly
256 effective in inhibiting 3-ABA conversion, with the $IC_{50}$ value of 3.5 μM (Table 2).

257 To further characterize the role of individual rat CYPs in 3-ABA metabolism and
258 to compare their efficiencies with those of human CYP enzymes, microsomes of
259 Baculovirus transfected insect cells (Supersomes$^{TM}$) containing recombinantly
260 expressed rat and human CYPs and NADPH:CYP reductase were utilized in an
261 additional part of this study.

262 First, a time-dependence of 3-ABA conversion catalyzed by rat recombinant
263 CYP1A1, 3A1 and 3A2, the enzymes found in the above experiments to be important
264 in 3-ABA metabolism in rat liver microsomes, and that of their human orthologous
265 CYPs (CYP1A1 and 3A4) was investigated. Whereas 3-ABA conversion catalyzed by
266 human and rat CYP1A1, rat CYP3A1 and human CYP3A4 is linear up to 20 minutes
267 of incubations, that by rat CYP3A2 is linear even up to 40 minutes of incubation (data
268 not shown). Because of linearity of 3-ABA conversion till 20 minutes, this time interval
269 was used in experiments evaluating the efficiency of individual human and rat CYPs
270 to metabolize 3-ABA.

271 All rat and human recombinant CYP tested in this study were effective to
272 metabolize 3-ABA (Figure 5). Among them CYPs of a 3A subfamily (CYP3A2 and

273 3A4/5) followed by CYP1A1 were the most efficient enzymes of both organisms
274 metabolizing 3-ABA. In addition, rat CYP2C11 and human CYP2A6, 2C19 and 2D6
275 were also effective in this reaction (Figure 5)
276
277 **DISCUSSION**

278 3-ABA, the human metabolite of the ubiquitous environmental pollutant 3-NBA,
279 was detected in the urine of smoking and nonsmoking salt mining workers
280 occupationally exposed to diesel emissions at similar concentration (1-143 ng/24 h
281 urine) to 1-aminopyrene (2-200 ng/24 h urine), the corresponding amine of the most
282 abundant nitro-polycyclic aromatic hydrocarbons detected in diesel exhaust matter
283 (Seidel et al. 2002). Comparison between experimental animals and human CYP
284 enzymes is essential for the extrapolation of animal toxicity data to assess human
285 health risk. Therefore, here we compared the ability of rat and human CYP enzymes
286 to metabolize 3-ABA.

287 The results found in the presented study have increased our knowledge on the
288 potential of human and rat CYP enzymes to metabolize 3-ABA and on the kinetics of
289 such reactions. Two experimental approaches were utilized in this study. For the first,
290 we used hepatic microsomal systems of rat found to be a suitable experimental
291 model (based on the fact that the same enzymes activate 3-ABA in human and rat
292 livers to form DNA adducts) (Arlt et al. 2004b; 2006c, Stiborova et al. 2006a).
293 Therefore, the results should provide some indications of what might occurs with 3-
294 ABA in livers of human. The second approach employed rat and human recombinant
295 CYP enzymes, which should mimic the enzymatic activity of actual CYP enzymes of
296 both organisms.

297 Recently, were have found that rat and human hepatic CYP1A1 and 1A2,
298 followed by human CYP2A6, are predominantly responsible for metabolic activation
299 of 3-ABA to form DNA adducts. The extrahepatic enzymes CYP1B1 and 2B6 were
300 also effective to activate 3-ABA, but to a much lower extent (Arlt et al. 2004b). On the
301 contrary, none of the other CYP enzymes such as CYP2C9, 2D6, 2E1 and 3A4
302 tested in this former study activated 3-ABA to species generating DNA adducts (Arlt
303 et al. 2004b). This finding corresponds to results found in the present study. We have
304 found that CYP1A enzymes are effective in oxidation of 3-ABA to the final oxidative
305 metabolite of this substance, 3-NBA. Namely, to the metabolite that is formed
306 through the formation of *N*-hydroxy-3-ABA. This reactive intermediate (N-hydroxy-3-
307 ABA) found in this study to be formed by CYP1A enzymes, is also decomposed to
308 the ultimate carcinogenic species of 3-ABA, nitrenium and/or carbenium ions, forming
309 the 3-ABA-derived DNA adducts (Figure 1). Hence, this metabolic activation pathway
310 seems to be mediated mainly by the CYP1A enzymes. On the contrary, 3-ABA
311 oxidation to further product(s) such as metabolite M18, which is supposed to be a
312 detoxification metabolite (Mizerovska et al. 2008), is catalyzed not only by CYP1A,
313 but also by other rat CYP enzymes. As follows from the studies on kinetics of 3-ABA
314 metabolism (presented study) as well as from the effects of selective inhibitors of
315 individual CYPs (Mizerovska et al. 2008 and presented study), beside CYP1A, the
316 CYP enzymes of 3A and 2C subfamilies might be very effective in such 3-ABA
317 metabolism in rat livers. Using rat recombinant CYPs, this suggestion was confirmed;
318 rat CYP3A2 followed by CYP1A1, 2C11 and 3A1 were the most efficient in 3-ABA
319 conversion. The results of this work also support our former findings showing a
320 suitability of the rat as an appropriate animal model to study the fate of 3-ABA in
321 humans (Arlt et al. 2004b; Stiborova et al. 2006a). Namely, here we have found that

322 human CYPs of a 3A subfamily (CYP3A and 3A5), orthologous CYPs to rat enzymes
323 mentioned above, followed by human and rat CYP1A1, are the most efficient in 3-
324 ABA metabolism.

325 In conclusion, the present study characterizing two metabolites formed from 3-
326 ABA by CYP-mediated oxidation, namely, a metabolite of 3-ABA, which is
327 responsible for generation of 3-ABA-derived DNA adducts (N-hydroxy-3-ABA) and its
328 final oxidation product (3-NBA), confirmed the participation of CYP1A enzymes in
329 metabolic activation of 3-ABA (Arlt et al. 2004b). Other rat and human CYP enzymes,
330 such a CYP3A and 2C are mainly important for detoxification metabolism of this
331 compound. Structural characterization of the major detoxification metabolite of 3-ABA
332 awaits further investigation.

333

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494 Table 1. Kinetic parameters of 3-ABA oxidation catalyzed by hepatic microsomal
495 CYPs

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| Parameters | | | | |
|---|---|---|---|---|
| Hepatic microsomes from rats pretreated with | $K_m$ | | $V_{max}$ | |
| | $K_{m1}$ [µM] | $K_{m2}$ | $V_{max1}$ [nmol 3-ABA min$^{-1}$ mg$^{-1}$] | $V_{max2}$ |
| β-naphtoflavone (CYP1A1/2) | 47.5 ± 0.5 | | 13.42± 0.134 | |
| Phenobarbital (CYP2B) | 60.7 ± 0.6 | | 16.8 ± 0.168 | |
| Control | 20.7 ± 0.2 | 417.5 ± 4.18 | 3.4 ± 0,3 | 48.5 ± 4.85 |

498
499 Experimental conditions are described in Material and methods, 3-ABA (5 - 50 µM)
500 and 0.5 mg microsomal protein were present in the incubation mixtures to determine
501 the $K_m$ and $V_{max}$ values. Values in the table are averages and standard deviations of
502 three determinations.

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520 Table 2. The effects of CYP inhibitors on 3-ABA oxidation by rat hepatic microsomes
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| Hepatic microsomes from rats pretreated with[a] | Inhibitor[b] | $IC_{50}$ [μM][c] |
|---|---|---|
| β-naphtoflavone (CYP1A1/2) | α-naphtoflavone (CYP1A1/2) | 10.9 ± 1.0 |
| β-naphtoflavone (CYP1A1/2) | Furafylline (CYP1A2) | 16.6 ± 1.6 |
| Untreated | Sulfaphenazole (CYP2C) | 10.8 ± 1.0 |
| Untreated | DDTC (CYP2E1) | 96.8 ± 6.1 |
| Untreated | Ketoconazole (CYP3A4) | 3.5 ± 0.3 |

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524 [a]Isoforms of CYPs induced by inducers are shown in brackets. [b]Isoforms of CYP
525 inhibited by selective inhibitors are shown in brackets. [c]Estimated from concentration-
526 dependent inhibition of 3-ABA oxidation by interpolation (inhibitors were 1 – 1000 μM
527 depending on the chemical). 3-ABA (20 μM) and 0.5 mg microsomal protein were
528 present in the incubation mixture. [d]Averages and standard deviations of three
529 determinations.
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Figure 1. Pathways of metabolism and DNA adduct formation of 3-NBA and 3-ABA. See text for details. NQO1, NAD(P)H:quinone oxidoreductase; NAT, *N*,*O*-acetyltransferases; SULT, sulfotransferase; PHS-1, prostaglandin H synthase-1 (cyclooxygenase 1); CYP, cytochrome P450; LPO, lactoperoxidase; MPO, myeloperoxidase; POR, NADPH:cytochrome P450 oxidoreductase; R = $-COCH_3$ or $-SO_3H$. R = $-COCH_3$ or $-SO_3H$; dA-$N^6$-ABA, 2-(2'-deoxyadenosin-$N^6$-yl)-3-aminobenzanthrone; dG-$N^2$-ABA, *N*-(2'-deoxyguanosin-$N^2$-yl)-3-aminobenzanthrone; dG-C8-*N*-ABA, *N*-(2'-deoxyguanosin-8-yl)-3-aminobenzanthrone.

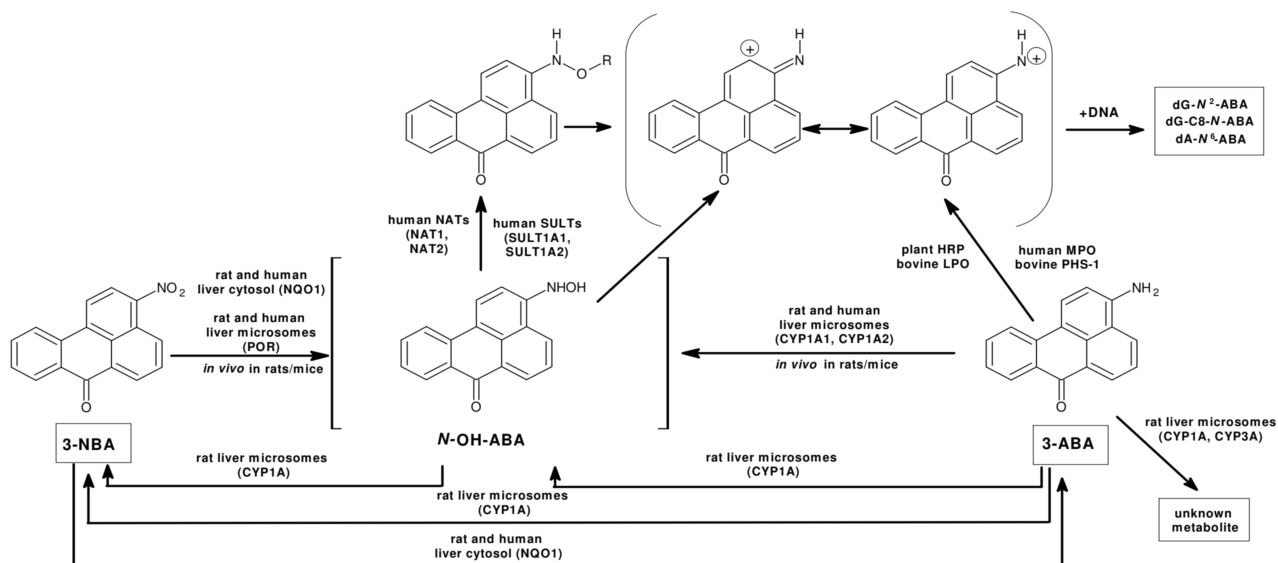


Figure 2. HPLC chromatographs of 3-ABA metabolites produced by hepatic microsomes of rats treated with β-NF (A), HPLC profiles of several standards; 3-ABA (B), *N*-hydroxy-3-ABA (C) and 3-NBA (D).

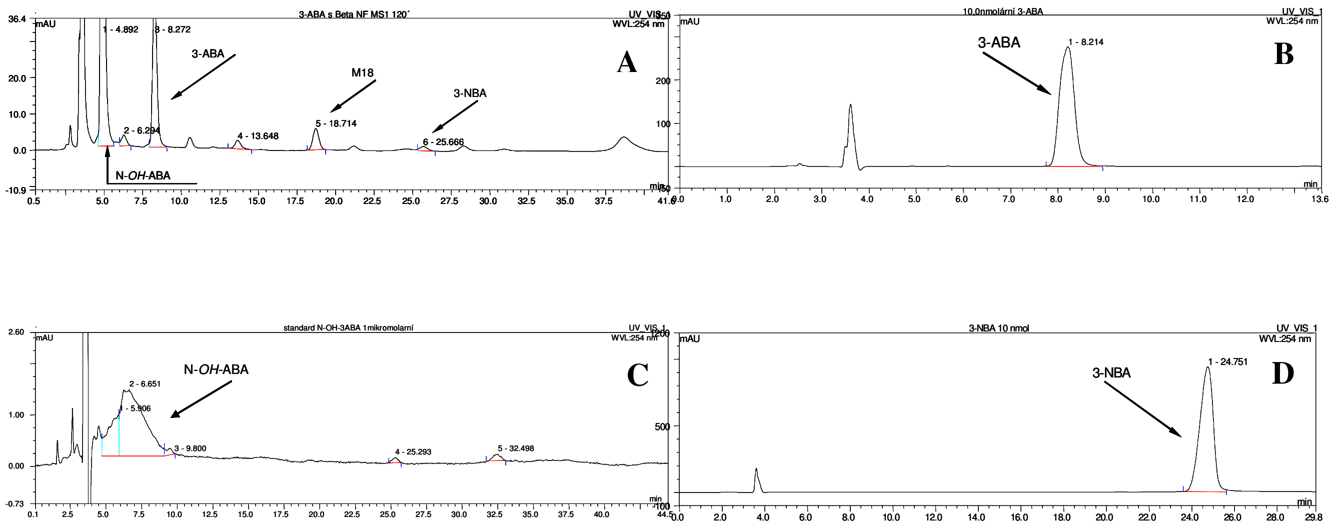
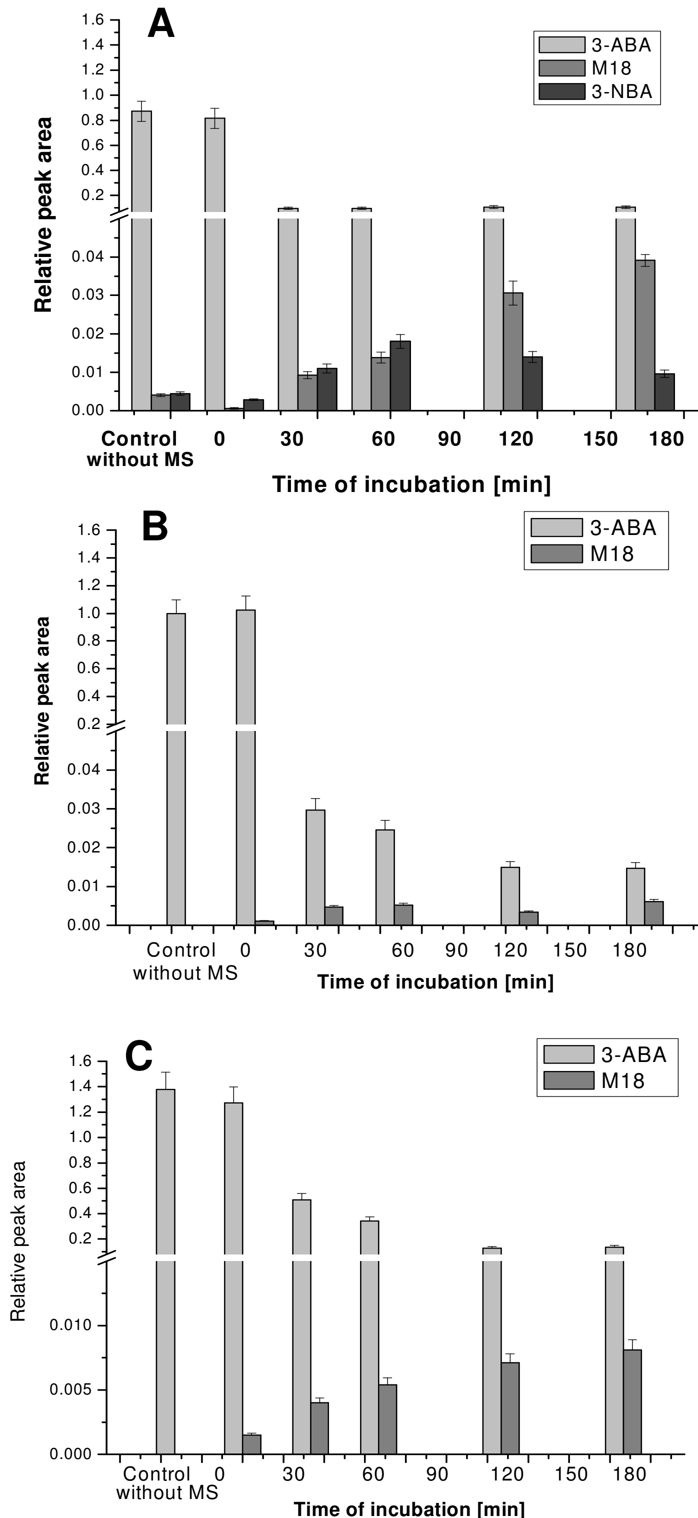


Figure 3. Time dependence of 3-ABA oxidation by hepatic microsomes of rats treated with β-NF (A), PB (B), and by those of control (uninduced) rats (C). Experimental conditions are described in Material and methods. Values of reaction rates of 3-ABA oxidation are averages and standard deviations of triplicate incubation. MS; microsomes



640 Figure 4. Double reciprocal plots of initial velocities of 3-ABA oxidation *versus*
641 concentrations of 3-ABA catalyzed by hepatic microsomes of rats treated with PB
642 (A), β-NF (B) and those of control (untreated) rats (C). Reaction rate (v) [nmol 3-
643 ABA/min/mg protein]. Experimental conditions are described in Material and
644 methods.

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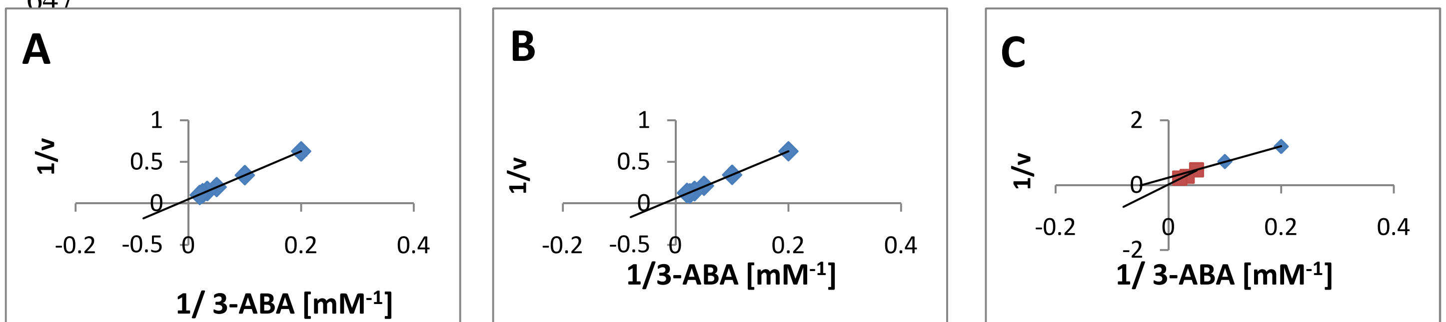


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684 Figure 5. Oxidation of 3-ABA by recombinant CYPs of rat (A) and human (B).
685 Experimental conditions are described in Material and methods. Values of reaction
686 rates of 3-ABA oxidation are averages and standard deviations of triplicate
687 incubation.

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