METABOLISM OF PIGMENT YELLOW 74 BY RAT AND HUMAN MICROSONAL PROTEINS

Yanyan Cui, Mona I. Churchwell, Letha H. Couch, Daniel R. Doerge, and Paul C. Howard

Division of Biochemical Toxicology (Y.C., M.I.C., L.H.C., D.R.D., P.C.H.) and National Toxicology Program Center for Phototoxicology (Y.C., L.H.C., P.C.H.), National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, Arkansas

Received December 10, 2004; accepted July 8, 2005

ABSTRACT:

Pigment Yellow 74 (PY74) (Fig. 1) is a monoazo-based pigment that is manufactured under a variety of trade names and formulations for various industrial applications including coatings, offset inks, dry toner, liquid inks, and paint finishes. PY74 and other monoazo pigments are not manufactured for consumption or direct dermal applications in humans and are not listed as an approved color additive for use in foods, drugs, cosmetics, or medical devices. PY74 is included in tattoo inks and permanent makeup that are available in Europe and the United States (Cui et al., 2004). Tattooing is an ancient art form in which colored materials are inserted into the skin with a sharp object. Recently, social/cultural trends in the United States and other developed countries have resulted in a surge in the popularity of tattooing. Tattooing has been identified as a significant risk factor for contraction of bacterial or viral (hepatitis and human immunodeficiency syndrome; HIV-AIDS) infections (Long and Rickman, 1994; Haley and Fischer, 2001; Hayes and Harkness, 2001; Hellard et al., 2004). In addition to infectious diseases, other risks associated with tattooing (reviewed in Papameletiou et al., 2003) have included acute phototoxicity, granulomatous and lichenoid reactions, development of pseudolymphomas, immunological-based rejection of the tattoo, and unwanted pigment spreading or inconsistencies within the tattoo. There have been reports of nonmelanoma skin cancer arising within tattoos (Weiner and Scher, 1987; McQuarrie, 1996; Jacob, 2002); however, these observations have not been substantiated by epidemiological studies.

The earliest tattoos were most likely derived from soot or charcoal with occasional inclusion of locally available minerals or plant products. In more recent times, inorganic compounds, such as titanium dioxide, mercuric oxide, and cadmium sulfide, have been used in tattoo inks (Lehmann and Pierchalla, 1988). The demand for an increasing number of color shades and color intensity by customers has resulted in a surge in the popularity of tattooing. Tattooing has been used recently as a permanent means to cover unwanted skin discoloration (e.g., vitiligo) or disfiguration (Mazza and Rager, 1993) or to correct skin color following some surgeries (Price et al., 2000); however, the single largest reason for voluntarily obtaining a tattoo has been personal artistic expression. In addition, permanent coloration of the lips and eyelids through tattooing is increasing in popularity and includes the application of black iron oxides and colored tattoo inks.

Tattooing has been identified as a significant risk factor for contraction of bacterial or viral (hepatitis and human immunodeficiency syndrome; HIV-AIDS) infections (Long and Rickman, 1994; Haley and Fischer, 2001; Hayes and Harkness, 2001; Hellard et al., 2004). In addition to infectious diseases, other risks associated with tattooing (reviewed in Papameletiou et al., 2003) have included acute phototoxicity, granulomatous and lichenoid reactions, development of pseudolymphomas, immunological-based rejection of the tattoo, and unwanted pigment spreading or inconsistencies within the tattoo. There have been reports of nonmelanoma skin cancer arising within tattoos (Weiner and Scher, 1987; McQuarrie, 1996; Jacob, 2002); however, these observations have not been substantiated by epidemiological studies.

The earliest tattoos were most likely derived from soot or charcoal with occasional inclusion of locally available minerals or plant products. In more recent times, inorganic compounds, such as titanium dioxide, mercuric oxide, and cadmium sulfide, have been used in tattoo inks (Lehmann and Pierchalla, 1988). The demand for an increasing number of color shades and color intensity by customers and reports of toxicity of some of the inorganic salts has led tattoo ink

ABBRVIATIONS: PY74, Pigment Yellow 74; PY74-M1, metabolite 1 of PY74; PY74-M2, metabolite 2 of PY74; BSA, bovine serum albumin; 3-MC, 3-methylcholanthrene; HPLC, high performance liquid chromatography; P450, cytochrome P450; PB, phenobarbital; MS, mass spectrometry; APCI, atmospheric pressure chemical ionization; FDA, Food and Drug Administration.
manufacturers to replace the inorganic salts with organic pigments to achieve the desired colors and eliminate the toxicity associated with certain metal salts. Lehmann and Pierchalla (1988) and Bäumler et al. (2000) have listed many of these pigments, which include yellow, orange, blue, green, and red pigments commonly used in the paint and commercial printing industries. Little or no toxicity data have been reported for many of these pigments, even though they contain structural alerts for mutagenicity and/or carcinogenicity.

PY74 is not listed as a hazardous substance, with an LD50 reported to be greater than 2000 to 5000 mg/kg in acute oral toxicity studies in rats, according to several Material Safety Data Sheets; however, one Material Safety Data Sheet does indicate that an irritation or allergic hazard exists. There have been no reports to date regarding the in vitro or in vivo metabolism or in vivo disposition of PY74. The primary objective of our study was to investigate the in vitro metabolism of PY74 by microsomal proteins and to identify the major metabolites for further evaluation of the safety of PY74 as part of our research program on tattoo ink safety.

Materials and Methods

Chemicals and Materials. PY74 [2-((2-methoxy-4-nitrophenyl)azo)-N-(2-methoxyphenyl)-3-oxybutanamide; CAS 6358-31-2; Color Index 11741], 3-nitroanisole, glucose-6-phosphate, glucose-6-phosphate dehydrogenase (type XXIV), NADP (sodium salt), NADH, bovine serum albumin (BSA), 3-methylxanthene (3-MC), dimethyl sulfoxide, anhydrous Na2SO4, and MgCl2 were purchased from Sigma-Aldrich (St. Louis, MO). Methanol and acetonitrile were high performance liquid chromatography (HPLC) grade and obtained from J. T. Baker (Phillipsburg, NJ). Water was obtained by ion-exchange to >18 MOHm and filtered at 0.1 μm (Modulab 2020 UF; US Filter Corp., Lowell, MA). All other reagents used were obtained at the highest purity from commercial sources.

Liver microsomes from male F344 rats pretreated with 3-MC (intraperitoneal administration in corn oil for 3 consecutive days, 25 mg/kg body weight/day) were prepared by a standard procedure in our laboratory (Howard et al., 1988). The microsomal pellets were suspended in 250 mM sucrose, 50 mM potassium phosphate, pH 7.4, at approximately 20 mg/ml and stored at ambient temperature (23°C) on a Luna C18 column (4.6 × 150 mm, 3-μm particle size) protected by a Luna C18 guard column (3 × 4 mm) (Phenomenex, Torrance, CA). The mobile phase flow rate was 800 μl/min and consisted initially of 70:15:15 water/methanol/acetonitrile, changing linearly to 5:47.5:47.5 over 50 min, and holding for 5 additional min. UV absorbance was monitored at 254 nm and injection volumes were 20 μl (see Fig. 2).

Liquid Chromatography-Mass Spectrometry. The HPLC separation coupled with mass spectrometry was performed with a column and mobile phase that differed from the conditions used in the analytical studies. The solid phase expressed CYP 1A1, 1A2, 1B1, 2B6, 2C9, 2D6, 2E1, and 3A4 (with cytochrome b5) were purchased from BD Biosciences Discovery Labware (Bedford, MA). The P450 concentration (1 nmol/ml) and protein concentrations of the expressed cytochromes were based on data supplied by the manufacturer and were as follows: CYP 1A1, 45 pmol P450/mg protein; CYP 1A2, 71 pmol P450/mg protein; CYP 1B1, 91 pmol P450/mg protein; CYP 2B6, 160 pmol P450/mg protein; CYP 2C9, 179 pmol P450/mg protein; CYP 2D6, 144 pmol P450/mg protein; CYP 2E1, 91 pmol P450/mg protein; CYP 3A4, 106 pmol P450/mg protein.

Microsomal Incubations. The PY74 stock solution was prepared immediately before the assay by mixing 0.4 ml of 1.3 mM PY74 in dimethyl sulfoxide with 10 ml of water containing 10 mg/ml bovine serum albumin. The coenzyme stock solution contained 2 mM NADP, 1.5 mM NADH, 20 mM glucose 6-phosphate, 2 units/ml glucose-6-phosphate dehydrogenase, 10 mM MgCl2, and 100 mM potassium phosphate, pH 7.4. Immediately before use, 1 mg/ml microsomal protein was added. Equal amounts (250 μl) of the two solutions were mixed at 37°C to start the reaction. Heat-inactivated microsomes (80°C for 5 min) were used in control samples. In studies investigating the dependence of PY74 metabolism on protein, PY74, or BSA concentration, the incubations were stopped at 20 min. In the time course studies, the incubations were stopped at 10, 20, 30, 45, 60, 90, and 120 min. The incubation was stopped by the addition of 3 ml of methanol/CH2Cl2 (1:2) with vigorous mixing. The CH2Cl2 was separated from the aqueous phase by centrifugation, the CH2Cl2 was collected, and the water phase was further extracted with 2 ml of CH2Cl2. After centrifugation, the organic phases were combined and 50 μl of internal standard 0.65 mM 3-nitroanisole in CH2Cl2 was added. The extract was dried using anhydrous Na2SO4 and evaporated in vacuo (SC210A Speed Vac Plus; Thermo Electron, Waltham, MA). The residue was reconstituted in 200-μl CH2Cl2 for HPLC analysis.

In the experiments to determine the specificity of human P450 isozymes in the metabolism of PY74, the incubations contained final concentrations of 0.25 nmol P450/ml, 1 mM NADP, 800 μM NADH, 10 mM glucose 6-phosphate, 1 unit/ml glucose-6-phosphate dehydrogenase, 4 mM MgCl2, 10 mg/ml BSA, 50 mM potassium phosphate, pH 7.4, and 25 μM PY74 (or the metabolite PY74-M1). The total volume was 200 μl and incubation time was 1 h at 37°C. The extraction and HPLC analysis were the same as described above for microsomal metabolism of PY74.

HPLC. HPLC analyses were carried out using a Waters liquid chromatography system (Waters, Milford, MA) consisting of an Alliance 2695 separation module and a 2996 photodiode array detector. The analyses were performed at ambient temperature (23°C) on a Luna C18 column (4.6 × 150 mm, 3-μm particle size) protected by a Luna C18 guard column (3 × 4 mm) (Phenomenex, Torrance, CA). The mobile phase flow rate was 800 μl/min and consisted initially of 70:15:15 water/methanol/acetonitrile, changing linearly to 5:47.5:47.5 over 50 min, and holding for 5 additional min. UV absorbance was monitored at 254 nm and injection volumes were 20 μl (see Fig. 2).

FIG. 1. Structure of PY74.

FIG. 2. HPLC separation of PY74 and its metabolites (PY74-M1 and PY74-M2) following incubation with 3-MC-induced rat liver microsomes. The column eluant was monitored for UV absorbance at 254 nm. The inset shows the UV-visible spectrum of PY74 and the two metabolites. The peak outlined by the dashed line at approximately 19.6 min is due to the absorbance of the internal standard.
MICROSOMAL METABOLISM OF TATTOO PIGMENT YELLOW 74

Results

Microsomal Metabolism of PY74. PY74 was incubated with rat liver microsomal protein from 3-MC-induced male F344 rat liver to determine whether PY74 could be metabolized. As shown in Fig. 2, two unidentified metabolites were separated by HPLC at 32.2 min (PY74-M2) and 35.1 min (PY74-M1) with UV-visible spectra similar to the spectrum of PY74. The inclusion of heat-inactivated microsomal protein or the omission of the NADPH-regenerating system did not result in the formation of these two products (data not shown). A metabolite eluting at 35.8 min was the major product following incubation of PY74 with PB-induced male Sprague-Dawley rat liver microsomal protein; however, the total formation of metabolites from untreated and PB-induced microsomes was approximately 10 times less than that from inclusion of 3-MC-induced microsomal protein. The internal standard 3-nitroanisole had a maximum absorbance at 330 nm, eluted at ~20 min under the same chromatographic conditions (Fig. 2), and was used in quantitative experiments.

PY74 has poor solubility in water. The solubility of PY74 in the microsomal incubations was increased by the inclusion of BSA. The metabolism of PY74 by 3-MC-induced microsomes plateaued between 5 and 10 mg/ml BSA (results not shown) and, therefore, we used 5 mg/ml BSA in subsequent studies.

The metabolic activity increased significantly when the concentration of microsomal protein was increased from 100 μg/ml to 500 μg/ml, plateauing at 500 μg/ml with no additional increase in metabolism at 1 mg/ml (data not shown). The dependence of PY74 metabolism on PY74 concentration indicated a linear dependence of metabolism on the concentration of PY74, plateauing at 50 μM. Our initial intent was to conduct zero-order studies to understand the kinetics of PY74 metabolism; however, no kinetic analyses were conducted because of the complexity of the analysis (i.e., dependent on combined Ks of PY74 from BSA directly into microsomal lipid, PY74 solubility in microsomal lipid, solubility of PY74 in aqueous solution, and Ks and Vmax properties of the P450).

The incubation conditions for the rat liver microsomal protein and expressed human P450s differed in the concentration of glucose 6-phosphate (10 and 5 mM, respectively) and BSA (5 and 10 mg/ml, respectively). The BSA concentration was adjusted in the human P450 studies in consideration of the low amount of P450 protein that was included in the assays. Since the studies with rat liver microsomal protein indicated that the metabolism plateaued between 5 and 10 mg/ml BSA, it was felt that the higher BSA concentration would not affect the qualitative results of the P450 assays. Similarly, since there was a low extent of P450 metabolism of both PY74 and PY74-M1, the reduced glucose 6-phosphate level in the P450 studies should not have affected the results.

The time course for the in vitro metabolism of PY74 by 3-MC-induced rat liver microsomal protein was investigated and is shown in Fig. 3. The loss of PY74 plateaued after 30 min with consumption of approximately 20% of the substrate. Since PY74-M1 and PY74-M2 had essentially the same UV-visible spectra (Fig. 2), and since we did not have a method for quantifying PY74-M1 and PY74-M2 (e.g., 3H- or 14C-labeled), we assumed that the molar extinction coefficients for PY74, PY74-M1, and PY74-M2 were equal. As a result, the HPLC eluate was monitored at 420 nm and the peak areas of all eluting compounds were quantified. The formation of PY74-M1 and PY74-M2 paralleled the loss of PY74, with a greater accumulation of PY74-M1 than PY74-M2.
Identification of Microsomal Metabolites. The two microsomal metabolites of PY74 (i.e., PY74-M1 and PY74-M2) were generated and purified as described under Materials and Methods. PY74-M1 was analyzed using HPLC coupled with negative-ion APCI mass spectrometry. PY74-M1 showed parent ion \([M-H]^-\) at \(m/z\) 401 (Fig. 4A), which is consistent with PY74-M1 being a hydroxylation product of PY74 (386 Da for PY74 + 16 Da for O). The major fragment ions obtained from PY74-M1 were \(m/z\) 236 and \(m/z\) 167, consistent with cleavage of the R=N−NH−R bond as shown in Fig. 4A, as has been previously shown for PY74 (Cui et al., 2004). Fractions of \(m/z\) 221 and 152 were either demethylation or deamination fragments from \(m/z\) 236 and 167, respectively. Taking into consideration the fragmentation of PY74 (Cui et al., 2004), the fragmentation of PY74-M1 is consistent with hydroxylation on the amino-anisole ring (C1′-C6′) and not on the amino-nitro-anisole ring (C1′-C6′).

The NMR spectrum of PY74-M1 was obtained and compared with the previously assigned spectrum of PY74 (Cui et al., 2004). Examination of the spectrum (not shown) for PY74-M1 revealed that all proton resonances from PY74 are present with the exception of the H4′ proton at 7.13 ppm (Table 1). The resonance for proton H3′ in PY74-M1 is shifted upfield by 0.46 ppm as compared with H3′ in PY74, suggesting hydroxyl substitution at an adjacent (ortho) carbon. H3′ in PY74-M1 has only a long-range meta-coupling of 2.7 Hz to H5′, whereas H3′ in PY74 has predominantly an ortho coupling of 8.1 Hz to H4′ and meta coupling to H5′. Similarly, the resonance for proton H5′ in PY74-M1 is shifted upfield by 0.56 ppm when compared with H5′ in PY74, suggesting hydroxyl ortho-substitution. The chemical shift for H6′ in PY74-M1 is 0.19 ppm upfield from H6′ in PY74, indicating that the 6-position is meta to the site of substitution on the ring. The resonances for H6′ and H5′ in PY74-M1 show ortho coupling constants (8.7 Hz). Taking into account these observations, we conclude that the site of hydroxylation on PY74-M1 is the C4′-position as shown in Fig. 4A. The appearance of a substantially broadened resonance at 4.94 ppm (Table 1) is attributable to the 4′-OH. The combined LC/MS and NMR analysis support that the structure of PY74-M1 is 2-((2-methoxy-4-nitrophenyl)azo)-N-(2-methoxy-4-hydroxyphenyl)-3-oxo-butanamide.

The addition of PY74-M1 to microsomal incubations containing 3-MC-induced rat liver microsomes indicated that PY74-M1 is metabolized to PY74-M2 (data not shown). The APCI negative-ion MS analysis of purified PY74-M2 is shown in Fig. 4B and indicates a parent ion \([M-H]^-\) at \(m/z\) 387, which is a loss of 14 Da from PY74-M1. The most probable structure of PY74-M2 to account for this mass is a demethylation product of PY74-M1. Fragmentation ions at \(m/z\) 152 and \(m/z\) 222 (Fig. 4B) suggest that the site of demethylation is the methoxy group on the amino-nitro-anisole ring (C1′-C6′) and not the amino-anisole ring (C1′-C6′).

The proton NMR spectrum of PY74-M2 confirmed the site of

![Fig. 4. The negative-ion LC/MS of PY74-M1 (panel A) and PY74-M2 (panel B).](image-url)

Table 1

<table>
<thead>
<tr>
<th>Position</th>
<th>PY74 (δ, ppm)</th>
<th>PY74-M1 (δ, ppm)</th>
<th>PY74-M2 (δ, ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-NH</td>
<td>11.65 (bs, 1H)</td>
<td>11.48 (bs, 1H)</td>
<td>11.48 (bs, 1H)</td>
</tr>
<tr>
<td>2-NH</td>
<td>14.74 (bs, 1H)</td>
<td>14.76 (bs, 1H)</td>
<td>15.09 (bs, 1H)</td>
</tr>
<tr>
<td>4</td>
<td>2.61 (s, 3H)</td>
<td>2.60 (s, 3H)</td>
<td>2.56 (s, 3H)</td>
</tr>
<tr>
<td>H3′</td>
<td>7.86 (d, 1H)</td>
<td>7.85 (d, 1H)</td>
<td>7.82 (d, 1H)</td>
</tr>
<tr>
<td>H5′</td>
<td>7.98 (dd, 1H)</td>
<td>7.97 (dd, 1H)</td>
<td>7.88 (dd, 1H)</td>
</tr>
<tr>
<td>H6′</td>
<td>7.78 (d, 1H)</td>
<td>7.77 (d, 1H)</td>
<td>7.48 (d, 1H)</td>
</tr>
<tr>
<td>7′</td>
<td>4.09 (s, 3H)</td>
<td>4.08 (s, 3H)</td>
<td>4.08 (s, 3H)</td>
</tr>
<tr>
<td>H3*</td>
<td>6.98 (dd, 1H)</td>
<td>6.52 (d, 1H)</td>
<td>6.51 (d, 1H)</td>
</tr>
<tr>
<td>H4*</td>
<td>7.13 (dt, 1H)</td>
<td>6.43 (dd, 1H)</td>
<td>6.43 (dd, 1H)</td>
</tr>
<tr>
<td>H5*</td>
<td>6.99 (dt, 1H)</td>
<td>6.43 (dd, 1H)</td>
<td>6.43 (dd, 1H)</td>
</tr>
<tr>
<td>H6*</td>
<td>8.41 (dd, 1H)</td>
<td>8.22 (d, 1H)</td>
<td>8.19 (d, 1H)</td>
</tr>
<tr>
<td>7′</td>
<td>3.97 (s, 3H)</td>
<td>3.93 (s, 3H)</td>
<td>3.93 (s, 3H)</td>
</tr>
<tr>
<td>4′-OH</td>
<td>4.94 †</td>
<td>4.94 †</td>
<td>4.94 †</td>
</tr>
</tbody>
</table>

† Substantially broadened resonance, assigned as -OH.
demethylation. PY74-M2 had the same number of proton resonances and a coupling pattern similar to that of PY74-M1, with the one notable exception that there was only a single methoxy resonance (integrated to approximately 3 hydrogens) at 3.93 ppm, indicating a loss of one of the methoxy groups (Table 1). The chemical shift of this methoxy group (3.93 ppm) was the same as that of the C7” methoxy in PY74-M1, which was only shifted 0.04 ppm upfield from the C7” group in PY74 (Table 1). The resonance of the methoxy group at C7’ (4.08 ppm in PY74-M1 and 4.09 ppm in PY74) was not present in PY74-M2. This finding suggested that PY74-M2 was the C7” O-demethylation product of PY74-M1. This assignment is further supported by a 0.29 ppm upfield shift for the H6’ proton, which is meta to the C7’ hydroxyl. Thus, the structure of PY74-M2 was identified as 2-((2-hydroxy-4-nitrophenyl)azo)-N-(2-methoxy-4-hydroxyphenyl)-3-oxo-butanamide. This metabolite is not stable in water at room temperature and decomposes to more polar compounds.

**Metabolism of PY74 and PY74-M1 by Human Cytochromes P450.** The incubation of PY74 with human liver microsomes (not shown) resulted in the formation of predominantly PY74-M1 and PY74-M2, with one minor peak that was less polar than PY74-M1, which was not identified due to lack of sufficient material. The specificity of the human P450 metabolism of PY74 is shown in Fig. 5, where CYP 1A2 had the highest activity, followed by CYP 1A1, 3A4, and 1B1. CYP 2B6, 2C9, 2D6, and 2E1 did not metabolize PY74. CYP 1A1 and CYP 1A2 metabolized PY74 to both PY74-M1 and PY74-M2 at a ratio of approximately 5.6:1, whereas CYP 1B1 formed exclusively PY74-M1 (data not shown).

CYP 1A1 and CYP 1A2 metabolized PY74-M1 to only PY74-M2 (Fig. 6). The other P450s did not metabolize PY74-M1.

**Discussion**

This is the first report describing the metabolism of PY74 by microsomal proteins or cytochromes P450 and the specificity of the site of metabolism (Fig. 7). This work is of public health significance because many individuals are being tattooed with this pigment as a color component of some yellow tattoo inks (Lehmann and Pierchalla, 1988; Bäumler et al., 2000; Cui et al., 2004). To date, neither the metabolism of PY74 nor the description of the identity of any metabolites of PY74 has been presented.

We find that PY74 is metabolized by microsomal protein from 3-MC-induced rat livers. 3-MC is an inducer of CYP1A proteins...
PY74 is a nitro-aromatic compound that contains an azo (or hydrazone; Cui et al., 2004) group. Many azobenzenes have been reported to be reduced to primary amines by P450s and NADPH-cytochrome P450 reductase (Levine, 1991). Metabolic azo reduction and cleavage is believed to be an activation reaction since the reduction products of some azo compounds exhibit toxic and mutagenic effects (Chung, 1983; Martin and Kennelly, 1985). A structure-activity study of various azobenzenes revealed that a polar electron-donating group (amino or hydroxyl) para to the azo linkage is required for substrate activity with microsomes. An electron-withdrawing group, such as a nitro group, on the benzoyl moiety prevents electron donation to the azo ring system, and the resulting product is inactive in azo reduction (Zbiaida, 1995). Although we did not examine the azo reduction of PY74 by skin microsomes or cytosol in our metabolic system, skin azo reduction of several azo compounds including 5-(phenylazo)-6-hydroxynaphthalene-2-sulfonic acid, Sudan I (1-phenylazo-2-naphthol; C.I. 12055), and Solvent Yellow 7 (4-phenylazophenol; C.I. 11800) has been demonstrated with a percutaneous absorption system (Collier et al., 1993). Therefore, the potential for azo reduction of PY74 by skin does exist and should be investigated. In addition, simulated solar light (Cui et al., 2004) and laser light used for tattoo removal (Vasold et al., 2004) have been shown to photochemically cleave PY74 at the azo group to generate aromatic amines, such as 2-methoxy-4-nitroaniline and 2-aminoanisole from PY74.

Under the definitions given in the Food Drug and Cosmetic Act, and associated regulations in 21 US Code of Federal Regulations Title 21, tattoo inks have been categorized by the Food and Drug Administration (FDA) to be professional use cosmetics. As a result, the pigments contained in the inks are considered to be color additives. Both cosmetics and color additives fall under the regulatory authority of the FDA. Although individuals are encouraged to report adverse events resulting from FDA-regulated products to the U.S. Food and Drug Administration, the lack of public perception that tattoo inks are regulated by the FDA probably explains why few complaints have been received, thus explaining why a comprehensive assessment or database on adverse events resulting from tattooing does not exist.

The results described in this paper show that tattoo ink pigments can be metabolized by phase I enzymes (P450s) that exist in the skin of rodents and humans. Anecdotal reports of tattoo fading are routinely heard from tattoo recipients who have yellow and orange tattoos. We suggest that the mechanisms for fading could include: 1) dispersion through the skin; 2) phagocytosis and removal; 3) metabolism of the pigments in the skin (this report); or 4) photochemical decomposition of the pigments as reported for PY74 (Cui et al., 2004; Vasold et al., 2004). No reports exist regarding the mutagenic, carcinogenic, or photocarcinogenic potential of PY74; however, 2-aminoanisole (o-anisidine) can be enzymatically activated in vitro to form DNA adducts (Stiborova et al., 2001, 2002) and is a urinary bladder carcinogen in mice and rats (National Toxicology Program, 1978). Oxidation of PY74-M1 could result in the formation of structure similar to a p-quinone imine, which is the active intermediate for o-anisidine (Stiborova et al., 2002) and acetaminophen (N-acetyl-p-benzoquinone imine; Rogers et al., 1997). To conduct a risk assessment for PY74, further studies on the purity of PY74 used in tattoo inks, the in vivo metabolism of PY74 in the skin, and quantification of the toxicity of its metabolites are among some of the studies that are warranted.

Acknowledgments. We thank F. E. Evans and F. A. Beland for the NMR analyses.
References

Address correspondence to: Dr. Paul C. Howard, Division of Biochemical Toxicology, HFT-110, National Center for Toxicological Research, U.S. Food and Drug Administration, 3900 NCTR Road, Jefferson, AR 72079. E-mail: Phoward@nctr.fda.gov

MICROSOMAL METABOLISM OF TATTOO PIGMENT YELLOW 74