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Photodecomposition of Pigment Yellow 74, a Pigment Used in Tattoo Inks¶

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ABSTRACT

Tattooing has become a popular recreational practice among younger adults over the past decade. Although some of the pigments used in tattooing have been described, very little is known concerning the toxicology, phototoxicology or photochemistry of these pigments. Seven yellow tattoo inks were obtained from commercial sources and their pigments extracted, identified and quantitatively analyzed. The monoazo compound Pigment Yellow 74 (PY74; CI 11741) was found to be the major pigment in several of the tattoo inks. Solutions of commercial PY74 in tetrahydrofuran (THF) were deoxygenated using argon gas, and the photochemical reaction products were determined after exposure to simulated solar light generated by a filtered 6.5 kW xenon arc lamp. Spectrophotometric and high-pressure liquid chromatography (HPLC) analyses indicated that PY74 photodecomposed to multiple products that were isolated using a combination of silica chromatography and reversed-phase HPLC. Three of the major photodecomposition products were identified by nuclear magnetic resonance and mass spectrometry as *N*-(2-methoxyphenyl)-3-oxobutanamide (*o*-acetoacetanisidide), 2-(hydroxyimine)-*N*-(2-methoxyphenyl)-3-oxobutanamide and *N,N*′-bis(2-methoxyphenyl)urea. These results demonstrate that PY74 is not photostable in THF and that photochemical lysis occurs at several sites in PY74 including the hydrazone and amide groups. The data also suggest that the use of PY74 in tattoo inks could potentially result in the formation of photolysis products, resulting in toxicity at the tattoo site after irradiation with sunlight or more intense light sources.

INTRODUCTION

Tattooing is an art form that has existed for several thousand years. References to tattooing can be found in ancient writings from Egypt, Mesopotamia, China, Japan and Oceania. Several reasons for voluntarily obtaining a tattoo include recreational expression, permanent makeup and medical–reconstruction purposes. Cosmetic reconstructive tattooing has been used to correct undesired features after traumatic injury to the skin (*e.g.* after burns or surgery) or to correct undesired skin conditions (*e.g.* vitiligo). In these cases, tattooing blends different skin color pigments into the skin to mask unwanted or missing features (1,2). Tattooing with India ink is also used internally for marking lesions for removal or for marking the locations of removed growths in colonoscopies, laparoscopies and laparotomies (3–8).

The reasons for obtaining recreational tattoos are multifaceted and include tribal or group affiliation, social identification and personal artistic expression. Tattooing has increased in popularity in the United States over the past decade (9–11), especially among youth (11–16). It has been estimated that as many as 20 million U.S. citizens have a recreational tattoo, which involves a significant portion of the population.

Tattooing is the method of using a needle or some other sharp object to force suspended colored solids through the epidermis into the dermis. Dermal deposition of the materials is important for tattoo longevity, and in humans, the tattoo pigment is typically deposited in the upper half to one-third of the dermis. The pigmented materials in the tattoo ink either remain in the interstitial space or are taken up by dermal cells through phagocytosis.

Two publications stand out as pivotal investigations into the materials used in modern tattoo inks. Lehmann and Pierchalla (17) examined the inorganic and organic materials used as pigments in tattoo inks. The inorganic pigments included CdSe, CdS, Cr_2O_3, $CoO–Al_2O_3$, Fe_2O_3, Fe_3O_4, HgS, TiO_2 (anatase and rutile) and ZnO. The organic pigments found in tattoo inks included Pigment Yellow 74 (PY74), Pigment Yellow 83 (PY83), Pigment Orange 16, Pigment Red 22 (PR22), Pigment Blue 15 (PB15), Pigment Violet 19 and Pigment Green 7 (PG7). Quantitative information

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Abbreviations: APCI, atmospheric pressure chemical ionization; FDA, Food and Drug Administration; HPLC, high-pressure liquid chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effects; PB15, Pigment Blue 15; PG7, Pigment Green 7; PR9, Pigment Red 9; PR22, Pigment Red 22; PR170, Pigment Red 170; PY74, Pigment Yellow 74; PY83, Pigment Yellow 83; THF, tetrahydrofuran; TLC, thin-layer chromatography.

concerning the concentrations of the pigments and diluents comprising the inks was not provided.

The other seminal article on the presence of organic pigments in tattoo inks was by Bäumler *et al*. (18) in 2000. Several tattoo inks were extracted, and the organic color component was determined by high-pressure liquid chromatography (HPLC) and mass spectrometry (MS) methods. The color pigments included Pigment Yellow 14, Pigment Yellow 55, PY74, PY83, Pigment Yellow 87, Pigment Orange 13, Pigment Red 5, Pigment Red 9, PR22, Pigment Red 112, Pigment Red 170 (PR170), Pigment Red 122, Pigment Violet 23, PB15, PG7 and Pigment Green 36.

A survey by the Danish Environmental Protection Agency (19) focused on tattoo inks (20). Several pigments that are not approved by the Danish government for inclusion in inks were detected and included Pigment Orange 36, PY74, PR170, Pigment Yellow 97, Pigment Red 146, Pigment Brown 25 and Pigment Red 266.

The U.S. Food and Drug Administration (FDA) has considered intradermal tattoos and permanent makeup to be cosmetics because they are applied to the body for the purpose of altering appearance or promoting attractiveness (21). There have not been any studies to date to determine the safety of the tattoo pigments under conditions of use, and as a result, no pigments are currently approved by the U.S. FDA for use in tattoo inks (21,22), although many pigments and colors have been approved by the U.S. FDA for use in foods, drugs and medical devices (21 CFR [U.S. Code of Federal Regulations] Parts 73, 74 and 82).

Bäumler *et al*. (23) reported that exposure of the monoazo pigment PR22 in acetonitrile to visible Nd:YAG laser light resulted in the formation of 2-amino-4-nitrotoluene. This has been followed by a recent report that Pigment Red 9 (PR9) likewise photodecomposes at the azo group in visible laser light to form aromatic amine compounds (24). The work of Bäumler suggests that exposure of tattooed individuals to the laser light sources used for tattoo removal may result in the formation of potentially mutagenic aromatic amine compounds at the tattoo site.

In view of the widespread practice of tattooing in youth, the photochemical photolysis of PR22 and PR9 with visible lasers and the unknown fate of photoactivated tattoo pigments, we have examined the previously unreported photochemical instability of PY74 after exposure to simulated solar light. PY74 was chosen for this study because of its commercial availability, simple structure and solubility when compared with other pigments.

MATERIALS AND METHODS

Reagents. PY74 (CAS 6358-31-2; CI 11741; 2-[(2-methoxy-4-nitrophenyl) azo]-*N*-(2-methoxyphenyl)-3-oxobutanamide), *o*-acetoacetanisidide (CAS 92-15-9) and tetrahydrofuran (THF) were obtained from Aldrich Chemical Company (Milwaukee, WI). The tattoo inks were obtained from commercial vendors as liquid suspensions and are identified by color designations. Methylene chloride was obtained from J. T. Baker (Phillipsburg, NJ), and methanol and acetonitrile were obtained from Fisher Scientific Co. (Pittsburgh, PA). All other reagents were of best available commercial grade.

Analysis of pigments in tattoo inks. Approximately 25 mg of tattoo ink was added to a 50 mL conical glass tube containing 2 mL of water. The contents were mixed vigorously and extracted three times with 15 mL of methylene chloride. The methylene chloride extracts were combined, dried *in vacuo* and reconstituted in 5 mL of methylene chloride. The samples were analyzed using a Varian (Walnut Creek, CA) HPLC system equipped with a Varian Pro-Star 330 diode array detector. The stationary phase was a Phenomenex (Torrance, CA) Prodigy 5 μm C_8 HPLC column (4.6 × 150 mm with 4.6 × 30 mm guard column), and the mobile phase started at 1:1 methanol and 0.015% formic acid and changed linearly to methanol in 25 min at a flow rate of 0.9 mL/min.

Exposure of PY74 to light. PY74 was dissolved in THF and placed in Teflon-capped cuvettes with a 2 mm light path (Starna Cells Inc., Atascadero, CA). Unless otherwise indicated, the cuvettes and samples were purged with argon before exposure to light. The light source was a 6.5 kW xenon arc lamp with the emission filtered through WG320 glass filters (0.6 mm; Schott Glass Technologies, Puryea, PA) to achieve a spectrum with ultraviolet light content consistent with terrestrial sunlight (UV-A:UV-B, ~21:1) (25). Cuvettes were typically placed 0.5 m from the source, which corresponds to an irradiance approximately equal to noontime terrestrial sunlight (summer at 34° latitude) with the following values: UV-C (250–280 nm), 2.2×10^{-6} W/cm^2; UV-B (280–315 nm), 2.4×10^{-4} W/cm^2; UV-A (315–400 nm), 5.2×10^{-3} W/cm^2; visible (400–800 nm), 4.2×10^{-2} W/cm^2.

Isolation of photoproducts. Larger scale preparations were used for the isolation of photoproducts for identification. PY74 was dissolved in argon-purged THF in either glass-capped cylinders or 500 mL glass bottles with Teflon-lined caps and placed at a distance of 0.4–0.5 m from the xenon arc light source until a color change was noted. The temperature in the cuvettes and vessels was maintained at <40°C using fans. The photodecomposition products were separated by column chromatography (2.5 × 30 cm silica gel, Merck grade 9385, 60Å, Aldrich, with methylene chloride as eluant), thin-layer chromatography (TLC; Alltech Associates [Deerfield, IL], silica 20 × 20 cm TLC plates with methylene chloride as eluant) or HPLC as described above.

Instrumentation. The HPLC systems consisted of a Varian 9012 HPLC pump equipped with either a Varian 9020 UV–VIS or a Pro-Star 330 diode array absorbance monitor. Injections were made manually using a Rheodyne 6125 injector. Absorption spectra of pigment solutions were obtained using a Perkin-Elmer Lambda 45 UV–VIS spectrometer *versus* a solvent blank. Mass spectra were obtained using a Quattro Ultima triple quadrupole mass spectrometer (Micromass, Manchester, UK) with an atmospheric pressure chemical ionization (APCI) interface, 400°C nebulizer probe and an ion source temperature of 120°C. Product ion spectra were obtained using a collision gas pressure of 1.8 mbar argon, a cone voltage of 40 V and various collision energies. The spectral irradiance of the simulated solar light source was determined using an Optronics OL-754 spectroradiometer (250–800 nm; Optronics Laboratories, Orlando, FL) equipped with a 6 inch integrating sphere and calibrated using a NIST (National Institute for Standards and Technology)-traceable quartz–tungsten–halogen standard (OL752-10E; Optronics Laboratories).

Nuclear magnetic resonance (NMR) spectra were obtained at 500 MHz using a Bruker AM-500 (Billerica, MA) operating at 28°C. Data were processed using either the spectrometer software (DISNMR) or the Bruker 1D WIN-NMR software on a PC. The samples were dissolved in CD_2Cl_2, and the residual $CDHCl_2$ peak from the solvent was assigned to 5.32 ppm as a reference. All reported NMR results are from first-order measurements. Chemical-shift assignments are described in the text. The "x" in Figs. 5 and 7 denotes an unidentified impurity.

RESULTS

Identification of PY74 in tattoo inks

PY74 had been reported to be present in tattoo inks obtained in Europe (17,18), and we tested for the presence of PY74 in yellow tattoo inks commonly used in the United States. Seven different yellow tattoo inks identified as Yellow, Dark Yellow, Sun Yellow, Mohawk Yellow, Canary Yellow, Tulip Yellow and Poppy were extracted as described in the Materials and Methods and analyzed by HPLC. PY74 was identified in six of the seven tattoo inks by comparison of the HPLC retention times and absorbance spectra of the eluting components with authentic PY74 (results not shown). The contribution of PY74 to the mass of each of the following tattoo inks was determined using PY74 standards (% wt/wt): Dark Yellow, 0.7%; Sun Yellow, 3.6%; Mohawk Yellow, 3.6%; Canary Yellow, 4.5%; Tulip Yellow, 10.5% and Poppy, 12.7%. PY74 was apparently the sole pigment in several of the inks; however, in several inks additional organic pigments were used in combination with PY74 to achieve the desired ink color. No attempt was made to identify inorganic pigments in the tattoo inks.

Characterization of PY74 by ^{1}H-NMR and MS

The ^{1}H-NMR spectrum of PY74 in CD_2Cl_2 is presented in Fig. 1. Many of the chemical shifts were assigned from the relative intensities, the coupling patterns and several simple decoupling experiments. The results are presented in Table 1 along with other NMR spectral parameters. The upfield region contains resonances from the three methyl groups. The methoxy proton resonances were assigned based on large nuclear Overhauser effects (NOE; 23% and 18%) to the adjacent *ortho*-substituted proton after saturation of each methoxy resonance. The resonance from the acetyl group is also distinguished from the methoxy resonances by its chemical shift. Two broadened singlets (linewidth *ca* 6.5 Hz) in the most downfield region are characteristic of exchangeable protons with strong hydrogen bonds, and the similar linewidths suggest that they both arise from amine protons.

PY74 could exist in several tautomeric forms, including at least two that contain an azo group and one that contains a hydrazone group. In one azo form there would be a methine proton at C2 and only a single strong intramolecular hydrogen bond; however, there was no NMR evidence of a methine resonance, and the chemical shifts greater than 11 ppm indicate two strong intramolecular hydrogen bonds. A more favorable azo tautomer contains a hydroxybutene group that would be stabilized by two strong intramolecular hydrogen bonds. One of the hydrogen bonds would involve an amine, but the second would involve a hydroxyl group, which would not be expected to produce the 6.5 Hz broadening mentioned above. The presence of two strongly hydrogen-bonded amine protons and no methine hydrogen is consistent with a hydrazone rather than an azo form.

Several NMR experiments were conducted to characterize the tautomeric form of PY74 in CD_2Cl_2. Small long-range coupling constants of up to 0.7 Hz (Table 1) were confirmed between each of the exchangeable protons and protons at the *ortho* and *meta* positions of the corresponding aromatic ring in resolution-enhanced spectra. These are analogous to benzylic coupling constants (26), except that the magnitudes of the couplings show small but not insignificant differences, as would be expected. They indicate that an amine proton is adjacent to each ring. From these data it was possible to assign the remaining resonances and to propose a predominant hydrazone tautomeric form that is stabilized by two strong intramolecular hydrogen bonds. In addition, saturation of the methoxy proton resonance at 4.09 ppm resulted in an NOE to the arylamine proton resonance at 14.74 ppm (1.7%), whereas no NOE was detected at the other arylamine resonance. Saturation of the methoxy proton resonance at 3.97 ppm had the opposite effect, with only the arylamine resonance at 11.68 ppm exhibiting a detectable NOE (1.8%). NOE resulting from saturation of each of the arylamine proton resonances were consistent with these results, although a small NOE was observed between the two arylamine protons (each at 0.4%). Other evidence for the hydrazone form came from detection of very small peaks, when recorded with higher signal to noise ratio (number of scans = 16 000), which are attributed to ^{15}N satellites. All expected ^{15}N satellites were detected with relative intensities consistent with the known natural abundance of ^{15}N. There was a slight isotope effect on chemical shift in the usual direction (upfield shift of 0.0006 ppm) for both ^{15}N-coupled protons. The ^{15}N-coupled resonances were much narrower because of the absence of ^{14}N quadrupole relaxation, and the magnitude of the $^1J_{N-H}$ coupling constants (98.4 Hz, 14.74 ppm; 90.6 Hz, 11.65 ppm) is characteristic of sp^2 arylamine protons (27).

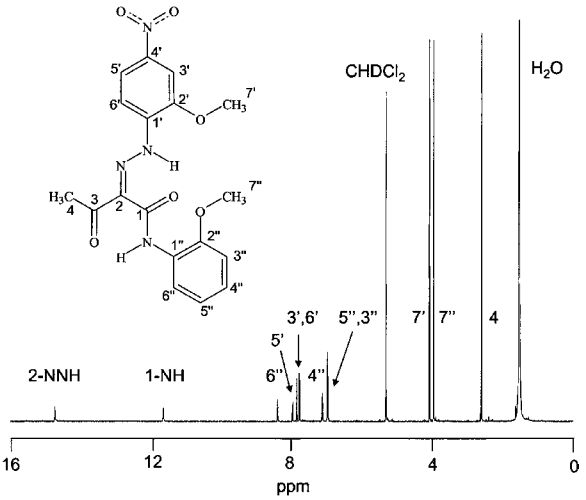

Figure 1. The 500 MHz ^{1}H-NMR spectrum of PY74 in CD_2Cl_2 is shown with resonance assignments.

PY74 was further characterized using APCI MS (Fig. 2). PY74 showed a molecular ion at *m/z* 387 in the positive-ion mode and a molecular ion at *m/z* 386 in the negative-ion mode (not shown). In the positive-ion mode, PY74 fragmented at the amide group, resulting in fragment ions of *m/z* 264 and 124 (the latter being identified as *o*-aminoanisole). Alternatively, PY74 fragmented at the azo group to form the fragment ions at *m/z* 167 and 169 ($R{=}NH^+$ or $R{-}NH_3^+$ of 2-amino-5-nitroanisole). Less prevalent was the fragmentation of PY74 between the C1–C2 bond giving rise to fragments of *m/z* 236 and 150. The fragment shown at *m/z* 369 results from loss of H_2O from PY74. Negative-ion fragmentation (not shown) showed a preference for azo bond cleavage (*m/z* 167) with further loss to *m/z* 152 (3-nitroanisole) or *m/z* 122 (nitrobenzene).

Photodecomposition of PY74

The exposure of PY74 to artificial sources of light is problematic because of the low solubility of PY74 in many solvents. We have found that PY74 is more soluble in THF and methylene chloride compared with other common solvents. Argon-purged solutions of PY74 in THF (approximately 40 μg/mL; ≈0.10 m*M*) were exposed to simulated solar light at 0.5 m for different lengths of time in 2 mm cuvettes as described in the Materials and Methods. The spectra of the solutions in the cuvettes were obtained and are shown in Fig. 3. PY74 has an absorption band with a maximum at 416 nm, and exposure to the light decreased the absorbance at a rate of approximately 0.42 AU/h under these conditions. During photodecomposition the maximum absorption band remained at 416 nm, indicating retention of a major chromophore in the visible portion of the spectrum. The absorption spectra did not form isobestic points, which indicated that the PY74 was photodecomposing into products that were also labile. The decrease in absorbance at 416 nm was accompanied by an increase in absorbance at 280–295 nm.

Samples of PY74 saturated in THF (≈0.25 m*M*) were exposed to simulated solar light and then subjected to analysis by HPLC to determine the amount of PY74 remaining and to investigate any decomposition products. The chromatography was monitored at 254 nm (Fig. 4). PY74 eluted at ~40 min and was essentially the only compound in the unexposed solution. At 0.5 and 1 h,

Table 1. [1]H-NMR spectral parameters for PY74 and photoproducts*

Position	PY74	Photoproduct "a" *N*-(2-methoxyphenyl)-3-oxobutanamide†	Photoproduct "b" 2-(hydroxyimino)-*N*-(2-methoxyphenyl)-3-oxobutanamide	Photoproduct "c" *N*,*N*″-bis(2-methoxyphenyl)urea	Photoproduct "d"	Photoproduct "e"	Photoproduct "f"
1-NH‡	11.65	9.06	11.37	7.12	9.26	9.38	9.40
2	—	3.57 (2H)	—	—	—	—	—
2-NNH‡	14.74	—	—	—	—	—	—
2-NOH‡	—	—	17.01	—	—	—	—
4	2.61	2.29	2.55	—	2.63	2.55	—
3′	7.86, J = 2.3	—	—	—	—	—	—
5′	7.98, J = 8.9, 2.3, 0.7	—	—	—	—	—	—
6′	7.78, J = 9.0, 0.3	—	—	—	—	—	—
7′	4.09	—	—	—	—	—	—
3″	6.98, J = 8.1	6.93	6.99, J = 8.1, 1.3	6.92, J = 8.0, 1.5	6.95, J = 8.1, 1.4	6.92, J = 8.1, 1.4	6.92, J = 8.1, 1.4
4″	7.13, J = 7.5, 1.6	7.06, J = 8.3, 7.4, 1.7	7.19, J = 8.3, 7.5, 1.6	7.01, J = 7.8, 7.5, 1.7	7.06, J = 8.1, 7.6, 1.6	7.08, J = 8.0, 7.8, 1.7	7.06, J = 8.0, 7.6, 1.7
5″	6.99, J = 7.9, 7.5, 1.3, 0.4	6.94, J = 1.4, 0.4	6.99, J = 1.3, 0.4	6.95, J = 7.9, 7.5, 1.6, 0.4	6.96, J = 7.9, 7.6, 1.4, 0.4	6.94, J = 7.8, 7.8, 1.4, 0.5	6.94, J = 7.8, 7.8, 1.4, 0.5
6″	8.41, J = 7.9, 0.4	8.28, J = 8.3, 1.6	8.25, J = 8.4, 1.6, 0.4	8.11, J = 7.8, 1.7	8.44, J = 7.9, 1.6	8.28, J = 8.0, 1.7	8.36, J = 8.0, 1.7
7″	3.97	3.91	3.96	3.89	3.94	3.89	3.90
Other	—	—	—	—	3.73 (2H), 2.87 (2H), 2.03 (2H)	4.93 (1H), 4.84 (1H), 3.83 (1H), 3.73 (1H), 1.8–2.0 (4H)	5.62 (1H), 3.98 (2H), 3.84 (1H), 3.72 (2H), 2.42 (1H), 1.6–1.9 (8H)

*Chemical shifts in parts per million followed by apparent measurable coupling constants in hertz as applicable. All values of 0.7 Hz or less are long-range coupling to the nearest amine proton. Number of protons are designated in parentheses in selected cases.

†Product "a" also known as *o*-acetoacetanisidide.

‡Broadened singlet in each case (see text).

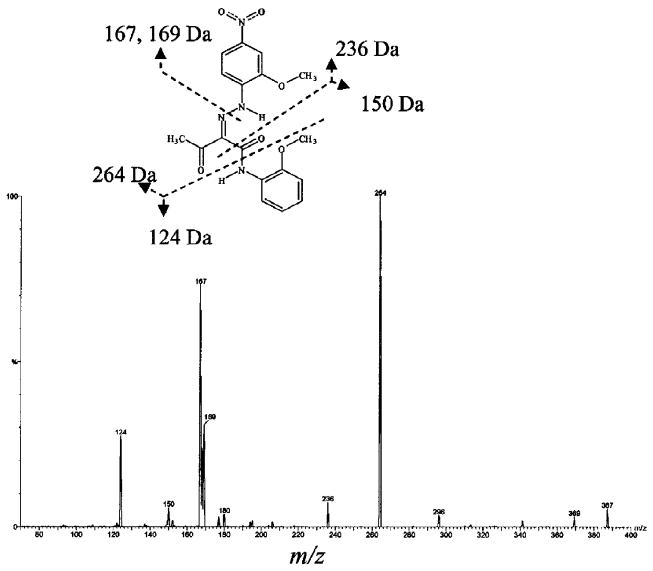

Figure 2. Positive-ion product mass spectrum of PY74 *m/z* 387 at a collision energy of 15 V.

photodecomposition products were observed and eluted with retention times between 32 and 37 min. Further exposure of the solution (Fig. 4) to simulated solar light resulted in the generation of products with decreased retention (*i.e.* increased polarity), with the largest HPLC peaks eluting between 13 and 18 min.

Isolation and identification of photodecomposition products

Exposure of PY74 to simulated solar light produced photodecomposition products that were separated by HPLC as shown in Fig. 4. The products from the 5 h exposure were initially isolated from PY74 using a silica gel column, with methylene chloride as the eluant. The fractions containing products less retentive than PY74 (the products that eluted between 13 and 18 min on HPLC) were then subjected to purification through repetitive injection and collection using HPLC. A total of 11 photolysis products were isolated, purified and subjected to NMR and MS analysis. Three of these photolysis products were unambiguously characterized. The peaks indicated by the "x" in Fig. 4 were not stable and tended to decompose primarily to Photoproducts "a" and "b" (data not presented).

Each of the stable photoproducts (a–f) was analyzed by ^{1}H-NMR spectroscopy, and the results are listed in Table 1. In each

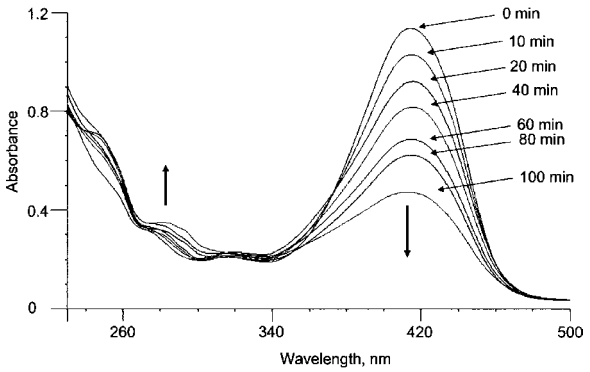

Figure 3. PY74 (≈0.1 m*M*) in THF (purged with argon) in 2 mm cuvettes was exposed to simulated solar light for the times indicated. The absorptivities of the solutions were determined from these spectra.

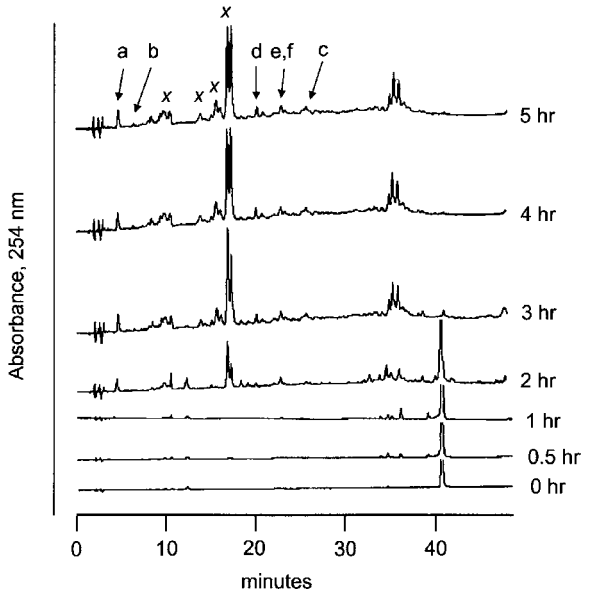

Figure 4. THF solutions saturated with PY74 (≈0.25 m*M*) and purged with argon were exposed to simulated solar light for up to 5 h. The solutions were analyzed by HPLC under conditions where PY74 eluted at ~40 min. The photoproducts that were isolated (a–f) are indicated in the figure, and those labeled "x" were unstable upon isolation.

case, all resonances from the aminoanisole ring were unambiguously detected and assigned. The spectral parameters are internally consistent with those of the corresponding ring in PY74. The amine proton resonance associated with this ring was found in all photoproducts, except that its chemical shift varied by more than 4 ppm. In most cases the small long-range couplings with the aromatic protons were confirmed by decoupling. No resonances from the nitrophenyl ring were detected in any of the products. Thus, all products likely have the same common structural element of a single aminoanisole moiety.

The NMR spectrum of Photoproduct "a" (Fig. 5) indicated the presence of a methylene group, and the acetyl group proton resonance was shifted upfield compared with PY74. This is consistent with the mass spectral data (Fig. 6), which indicated that the product was *N*-(2-methoxyphenyl)-3-oxobutanamide. The 2.6 ppm upfield shift for the amine proton compared with PY74 reflects a weakening of the intramolecular hydrogen bond due to the reduction in planar character. The proposed compound is available commercially as *o*-acetoacetanisidide and its HPLC retention and NMR spectrum (not shown) matched that of Photoproduct "a."

Examination of the structure of Photoproduct "a" by MS in a positive-ion mode revealed a protonated molecule at *m/z* 208 (Fig. 6). The positive-ion fragment ion at *m/z* 190 is consistent with the loss of water from *o*-acetoacetanisidide. Fragmentation of the C1–C2 bond resulted in the *m/z* 150 fragment, whereas amide bond breakage resulted in the *m/z* 124 fragment. As a result, the MS supports that Photoproduct "a" is *N*-(2-methoxyphenyl)-3-oxobutanamide (*o*-acetoacetanisidide).

The NMR spectrum of Photoproduct "b" is shown in Fig. 7. The most downfield part of the spectrum contains two resonances that each integrate as a single proton. The resonance assigned to C1–NH has a chemical shift similar to that in PY74, suggesting retention of the hydrogen bonding. The other resonance (17.01 ppm), however, is

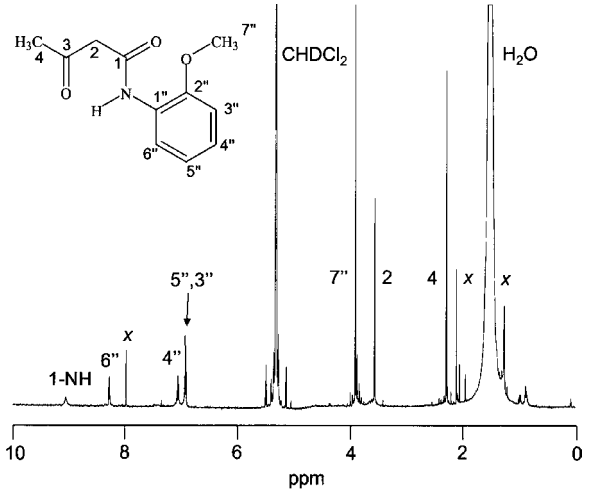

Figure 5. The 500 MHz ^{1}H-NMR spectrum of Photoproduct "a" in CD_2Cl_2 is shown with resonance assignments.

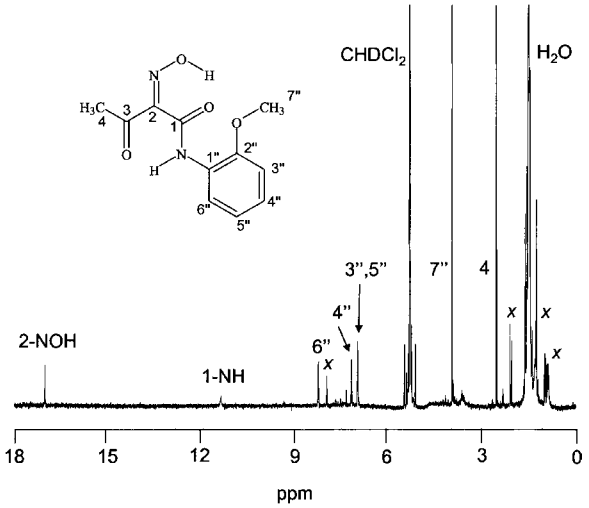

Figure 7. The 500 MHz ^{1}H-NMR spectrum of Photoproduct "b" in CD_2Cl_2 is shown with resonance assignments.

well downfield of that in PY74, is less broadened (linewidth *ca* 2.4 Hz) and is consistent with a hydrogen-bonded oxime.

Mass spectral analysis of Photoproduct "b" (Fig. 8) was used to confirm the structure as 2-(hydroxyimine)-*N*-(2-methoxyphenyl)-3-oxobutanamide. The product had a protonated molecule of *m/z* 237 in the positive-ion mode. The two primary fragmentation products were from breakage of the C1–C2 bond to a product with *m/z* 150 or loss of the primary amine and acetyl group to yield a product at *m/z* 177. Minor products included fragmentation of the acetyl group (*m/z* 195) or cleavage of the amide bond (*m/z* 122). The mass spectrometric data are fully consistent with 2-(hydroxyimine)-*N*-(2-methoxyphenyl)-3-oxobutanamide as the structure of the second photoproduct.

The NMR spectral analysis of Photoproduct "c" indicated that the amine resonance at 7.12 ppm was well upfield of that of the other photoproducts and of PY74 (Table 1, Fig. 9). This is consistent with a structure that has lost the intramolecular hydrogen bond. No acetyl protons were detected, and no other protons aside from the usual proton resonances from the aminoanisole fragment were detected.

MS was used to assign the structure of Photoproduct "c." A full-scan mass spectrum confirmed the protonated molecule at *m/z* 273 because a M + Na was detected at *m/z* 295. Fragmentation of the *m/z* 273 protonated molecule led to products at *m/z* 150, *m/z* 124 and *m/z* 109 (Fig. 10). The fragmentation pattern, consistent with the NMR analysis, supports the structure of Photoproduct "c" as *N*,*N*′-bis(2-methoxyphenyl)urea as shown in Fig. 10. This structure is additionally supported by negative-ion MS (data not shown).

Mass spectrometric examination of the final three purified photoproducts (d–f, Fig. 4) indicates parent masses greater than PY74 in increments consistent with the addition of THF, suggesting the participation of THF in the photodecomposition and incorporation of all or some of the solvent molecule in the photoproduct. NMR analysis likewise indicates the addition of aliphatic protons, and the aminoanisole fragment was present in each case (Table 1). We were unable to identify these secondary products.

DISCUSSION

In this report, we have shown that PY74 is present in tattoo inks obtained from commercial sources within the United States. The presence of PY74 in U.S. tattoo inks and the presence of PY74 in

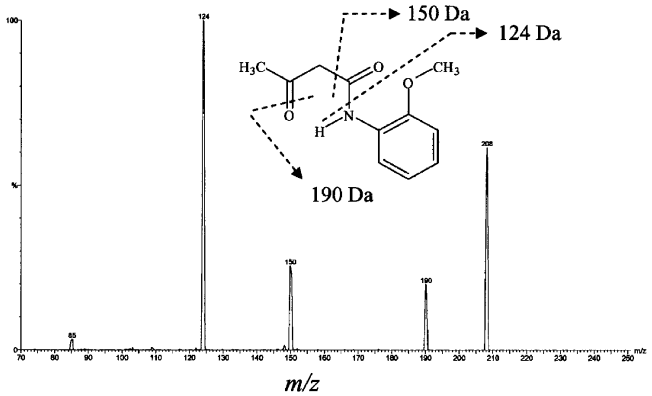

Figure 6. Product ion mass spectrum (*m/z* 208) of Photoproduct "a" in the positive-ion mode is shown. The product ions were obtained in positive-ion mode at a collision energy of 10 V.

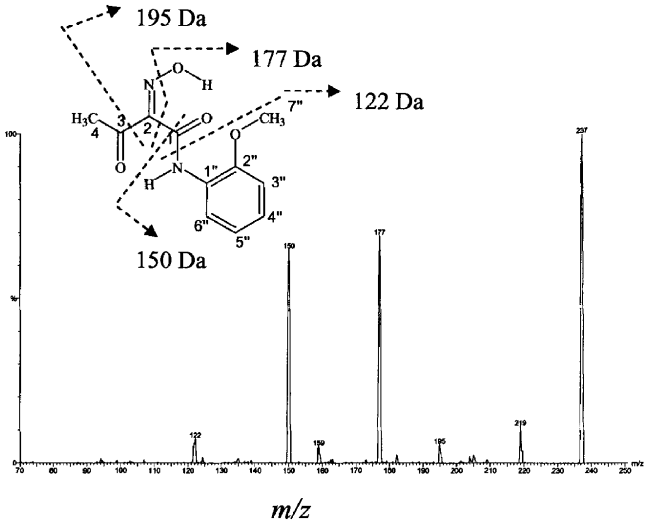

Figure 8. Product ion mass spectrum (*m/z* 237) of Photoproduct "b" in the positive-ion mode is shown. The product ions were obtained in positive-ion mode at a collision energy of 10 V.

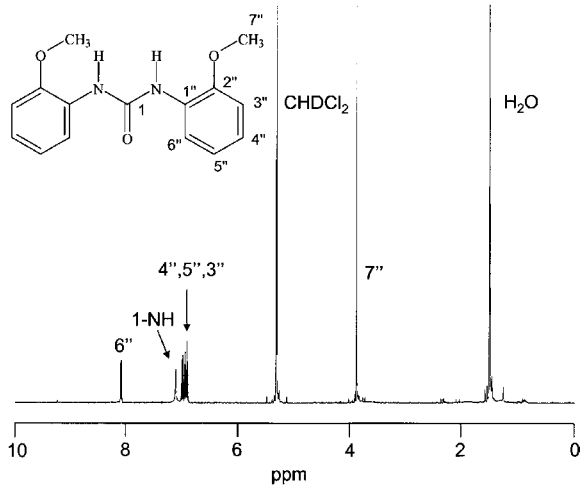

Figure 9. The 500 MHz ^{1}H-NMR spectrum of Photoproduct "c" in CD_2Cl_2 is shown with resonance assignments.

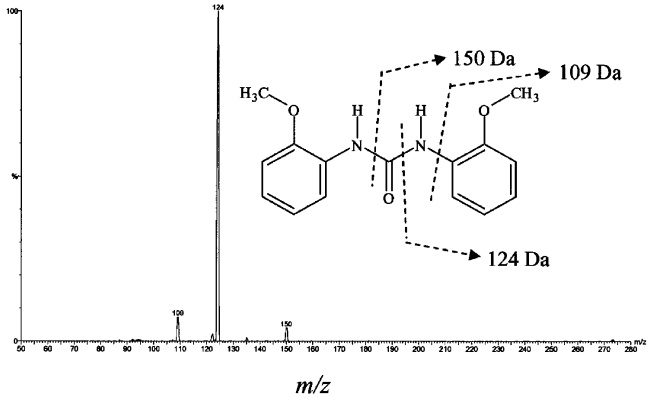

Figure 10. Positive-ion product mass spectrum of Photoproduct "c" (*m/z* 273) with a collision energy of 20 V.

tattoo inks obtained in Europe (17,18) suggest that PY74 is a commonly used pigment in tattoo inks.

The NMR spectroscopic analyses of PY74 in CD_2Cl_2 indicate that PY74 exists predominantly in a hydrazone form. Tautomerism plays an important role in the chemical character of many monoazo compounds (28). The physical–chemical properties of many aromatic azo compounds have been reported and used to determine their tautomeric equilibria in different solvents and the role of tautomerism in excited-state decay. For instance, Sahu *et al.* (29) reported that the tautomerism between the azo and hydrazone forms of 2-[(2-hydroxy-4-methyl)azo]benzoic acid results in a spectral shift of 100 nm (azo = 380 nm; hydrazone = 480 nm) and is dependent on the solvent, pH and temperature. With 2-[(2-hydroxy-4-methyl)azo]benzoic acid, addition of base facilitates tautomerism between the azo form and the hydrazo form by allowing the *ortho*-hydroxy hydrogen to hydrogen bond to the azo nitrogen. In a study using 1-phenylazo-2-naphthol and 2-phenylazo-1-naphthol, Joshi *et al.* (30) reported that photoexcitation of the azo form results in excited-state intramolecular proton transfer to the excited hydrazone form, which then undergoes fluorescence decay to the ground state. Any tautomerism in PY74 between the azo and hydrazone forms would not involve loss of aromaticity, as with the azobenzenes or azonaphthols mentioned above, where the azo tautomer would be favored. Other azoacetoacetanilides such as Pigment Yellow 1 (also known as Hansa Yellow G) or bis-azoacetoacetanilides such as Pigment Yellow 12 and 13 exist exclusively in the hydrazone form, being stabilized through the formation of intramolecular hydrogen bonding (28). As a result, the observation that PY74 exists primarily in the hydrazone form is consistent with these previous observations.

In this study we demonstrate that PY74 dissolved in THF photodecomposes in the presence of simulated solar light. PY74 has no appreciable absorbance between 250 and 350 nm and a maximum absorption band at 416 nm, suggesting that either visible light or fluorescent tubes emitting UV-A light could initiate the photodecomposition of this pigment. This study was designed to identify the products after photochemical decomposition of PY74 in THF and was not designed to imitate the conditions that would exist for a tattoo pigment in the skin where oxygen would be present and the solid pigment could be in either an aqueous or a lipid environment. Investigation of the precise mechanism or efficiency of this photochemical process was beyond the scope of this study; however, our data suggest some insights into the mechanism of photochemical decomposition of PY74.

The excitation of an electron in PY74 results in a Type-I photochemical reaction, resulting in either the addition of PY74 to a hydrogen source (THF) or the photolysis of PY74. The first products that are formed after illumination of PY74 in THF, eluting between 32 and 39 min on HPLC (Fig. 4), decomposed upon isolation but were examined by online HPLC/MS-MS (data not presented) and had molecular weights equal to PY74 plus THF. The persistence of these products with time (Fig. 4) suggests that they are not precursors to the other photoproducts. The isolation of *o*-acetoacetanisidide and 2-(hydroxyimine)-*N*-(2-methoxyphenyl)-3-oxobutanamide as photodecomposition products of PY74 suggests that the bonds adjacent to the hydrazone are particularly susceptible to photochemical photolysis. This is consistent with the observations reported by Bäumler *et al.* (23,24), where the laser-induced photolysis of PR22 and PR9 occurred at the azo groups, generating 2-amino-4-nitrotoluene with PR22.

It is also possible that PY74 could undergo a Type-II photochemical reaction where irradiation and intersystem crossover results in an excited triplet state, which then transfers the energy to oxygen, generating singlet oxygen ($^1O_2$). The rate of photodecomposition of PY74 in THF is greatly reduced in the presence of oxygen (data not presented). This suggests that under conditions where oxygen is present, a Type-II mechanism competes with the Type-I photochemical lysis. Determination of the quantum efficiency of PY74 photolysis and the kinetics of $^1O_2$ generation was beyond the scope of this initial study; however, because of the potential of $^1O_2$ to cause cytotoxic and genotoxic events *in vivo,* the potential of the Type-I photolysis products of tattoo inks and Type-II–generated $^1O_2$ to cause toxicity in the skin of tattooed individuals should be explored.

The NMR analysis of the PY74 used in this study showed the presence of about 0.4% *o*-acetoacetanisidide and 1% 2-(hydroxyimine)-*N*-(2-methoxyphenyl)-3-oxobutanamide (molar percentages). The manufacture of PY74 would most likely occur through diazotization of 2-amino-5-nitroanisole to the corresponding diazonium salt and condensation of the diazonium salt with *o*-acetoacetanisidide. This suggests that the purity of PY74 used in the manufacture of tattoo inks is not certain, and the extent of contamination and toxicity of the PY74 used in these and other products should be explored. An additional complication that

should be considered is the presence of inorganic materials such as titanium dioxide in many inks (17,31).

There has been a dramatic change in the cultural acceptance of tattooing in the United States in recent years. In the past, tattooing was primarily restricted to small subsets of the population. Tattooing has moved into mainstream culture during the past decade, with widespread acceptance and practice among U.S. youth. Tattoos in the mid-1900s were essentially black or blue, with the occasional color coming from inorganic compounds such as oxides of iron and mercury. As reported by Lehmann and Pierchalla (17), by Bäumler *et al.* (18,24) and in this article, monoazo and bisazo dyes are being used as pigments in modern tattoo inks. The toxicological safety of these materials in the absence or presence of sunlight has not been evaluated.

Tattooing has not been without risk and consequences and historically has included (1) infection or contraction of disease; (2) dermatological reactions, some of which may be severe or other significant allergic reactions to chemicals in the tattoo; and (3) complications after removal of the tattoo. Perhaps the greatest potential risk from tattooing is the spread of diseases, *e.g.* bacterial infections, hepatitis and HIV infections (32), through improperly cleaned or sterilized needles and equipment (33–39). In the United States, the practice of tattooing is regulated in most states through the state health departments (40), where a major concern is to practice proper hygiene in order to minimize the spread of diseases. The U.S. FDA and other agencies have posted Web sites for consumers regarding the safety and regulation of tattooing (22,41).

The second potential risk from tattooing is dermatological reaction to ingredients in the tattoo. There have been numerous reports of adverse reactions to tattoos (42–45), especially after exposure of the tattoos to sunlight (32,46–50). Cadmium sulfide has been used as a yellow-orange–colored pigment in the past in some tattoos and has been reported to induce severe allergic responses in skin after exposure to sunlight (47). However, tattoos containing red colors are most frequently associated with skin disorders (48–60). In addition, there have been reports of basal or squamous cell carcinomas and melanomas arising within tattoos (61–67), suggesting direct chemical or photochemical participation in the etiology of these diseases. Sangueza *et al.* (68) reported on a patient who developed multiple pseudolymphomas at a tattoo site, which progressed for a period of 4 years into malignant and monoclonal B-cell large-cell lymphoma. The possibility that tattoo pigments elicit adverse biological responses that could lead to cancer growth is intriguing and anecdotally supported by clinical observations; however, because no epidemiological studies have been conducted to date to examine the role of tattooing in skin cancer, the participation of tattooing or tattoo pigments in the etiology of any skin disease remains unclear and unsubstantiated.

The third potential risk from tattooing is the process of removal of the tattoo. Tattoos can be removed by dermabrasion, salabrasion, surgical removal of the skin or laser treatment (16,69,70). Each of these processes has procedural advantages and disadvantages including granuloma formation, keloid formation, unwanted color change and incomplete removal (69,71–73).

In this article, we present new evidence that an additional potential risk associated with tattoos may be from the toxicity of photochemical products of the tattoo pigments. The first report of this potential toxicity came from the work of Bäumler *et al.* (23), in which they reported that exposure of PR22 in acetonitrile to 532 nm Nd:YAG laser light resulted in the formation of 2-methyl-5-nitroaniline, a known carcinogen. PR22 is a monoazo aromatic pigment with many similarities to PY74. Bäumler *et al.* (24) recently extended this work and obtained similar results with PR9. Their report and our studies suggest that photochemical activation of tattoo pigments *in situ* during sunlight exposure, tanning or laser removal may generate toxic products. Although the light intensity inside tattooed skin will differ from that of the incident light on the surface of the skin, the potential for photodecomposition of the tattoo pigment still exists.

In summary, our study suggests that the use of PY74 as a pigment in tattoo inks may result in an increased risk of toxicity at the tattoo site from photodecomposition products of PY74, contaminating impurities in the pigments, or Type-II photochemical generation of $^1O_2$. Studies are in progress to address the toxicity of PY74 *in vivo* in mice tattooed with PY74 and exposed to simulated solar light.

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REFERENCES

1. Mazza Jr., J. F. and C. Rager (1993) Advances in cosmetic micropigmentation. *Plast. Reconstr. Surg.* **92**, 750–751.
2. Mutalik, S. and A. Ginzburg (2000) Surgical management of stable vitiligo: a review with personal experience. *Dermatol. Surg.* **26**, 248–254.
3. Lane, K. L., R. Vallera, K. Washington and M. R. Gottfried (1996) Endoscopic tattoo agents in the colon. Tissue responses and clinical implications. *Am. J. Surg. Pathol.* **20**, 1266–1270.
4. Nizam, R., N. Siddiqi, S. K. Landas, D. S. Kaplan and P. G. Holtzapple (1996) Colonic tattooing with India ink: benefits, risks, and alternatives. *Am. J. Gastroenterol.* **91**, 1804–1808.
5. Shatz, B. A., L. B. Weinstock, P. E. Swanson and E. P. Thyssen (1997) Long-term safety of India ink tattoos in the colon. *Gastrointest. Endosc.* **45**, 153–156.
6. McArthur, C. S., S. Roayaie and J. D. Waye (1999) Safety of preoperation endoscopic tattoo with India ink for identification of colonic lesions. *Surg. Endosc.* **13**, 397–400.
7. Lee, J. G., A. H. Low and J. W. Leung (2000) Randomized comparative study of indocyanine green and India ink for colonic tattooing: an animal survival study. *J. Clin. Gastroenterol.* **31**, 233–236.
8. Price, N., M. R. Gottfried, E. Clary, D. C. Lawson, J. Baillie, K. Mergener, C. Westcott, S. Eubanks and T. N. Pappas (2000) Safety and efficacy of India ink and indocyanine green as colonic tattooing agents. *Gastrointest. Endosc.* **51**, 438–442.
9. Brown, K. M., P. Perlmutter and R. J. McDermott (2000) Youth and tattoos: what school health personnel should know. *J. Sch. Health* **70**, 355–360.
10. Drews, D. R., C. K. Allison and J. R. Probst (2000) Behavioral and self-concept differences in tattooed and nontattooed college students. *Psychol. Rep.* **86**, 475–481.
11. Marcoux, D. (2000) Appearance, cosmetics, and body art in adolescents. *Dermatol. Clin.* **18**, 667–673.

12. Ceniceros, S. (1998) Tattooing, body piercing, and Russian roulette. *J. Nerv. Ment. Dis.* **186**, 503–504.
13. Greif, J. and W. Hewitt (1998) The living canvas. *Adv. Nurse Pract.* **6**, 26–31.
14. Armstrong, M. L., Y. Masten and R. Martin (2000) Adolescent pregnancy, tattooing, and risk taking. *MCN Am. J. Matern. Child Nurs.* **25**, 258–261.
15. Armstrong, M. L., K. P. Murphy, A. Sallee and G. Watson (2000) Tattooed army soldiers: examining the incidence, behavior, and risk. *Mil. Med.* **165**, 135–141.
16. Anderson, R. R. (2001) Regarding tattoos: is that sunlight, or an oncoming train at the end of the tunnel? *Arch. Dermatol.* **137**, 210–212.
17. Lehmann, G. von and P. Pierchalla (1988) Tätowierungsfarbstoffe. *Derm. Beruf Umwelt* **36**, 152–156.
18. Bäumler, W., E. T. Eibler, U. Hohenleutner, B. Sens, J. Sauer and M. Landthaler, (2000) Q-switched laser and tattoo pigment: first results of the chemical and photophysical analysis of 41 compounds. *Lasers Surg. Med.* **26**, 13–21.
19. Danish Environmental Protection Agency (2003) Chemicals in Consumer Products. Available at: http://www.mst.dk/chemi/01080000.htm. Accessed on 1 June 2004.
20. Danish Environmental Protection Agency (2003) Survey No. 2: Investigation of Pigments in Tattoo Colours, January 2002. Available at: http://www.mst.dk/chemi/01080200.htm. Accessed on 1 June 2004.
21. Food and Drug Administration (1994) Tattoos. *FDA Medical Bulletin.* **24**, 8. Published 8 May 1994, by the U.S. Department of Health and Human Services, Public Health Service, Food & Drug Administration, Rockville, MD.
22. FDA Center for Food Safety and Applied Nutrition (2004) Tattoos and Permanent Makeup. Available at: http://www.cfsan.fda.gov/~dms/cos-204.html. Accessed on 1 June 2004.
23. Bäumler, W., R. Vasold, N. Naarmann, D. Fischer, B. Sens, B. König and M. Landthaler (2002) Q-switch laser and tattoo pigments—a chemical analysis of laser induced decomposition products. *Am. Soc. Laser Med. Surg. Abstr.* **2002**, 78.
24. Bäumler, W., R. Vasold, B. König and M. Landthaler (2004) Q-switched laser and tattoo pigments, a chemical analysis of laser induced decomposition compounds. *Toxicologist* **78**(S1), 1079.
25. Howard, P. C., R. L. Sams II, J. R. Bucher and W. T. Allaben (2002) Phototoxicology and photocarcinogenesis at the U. S. Food and Drug Administration's National Center for Toxicological Research. *J. Food Drug Anal.* **10**, 252–257.
26. Evans, F. E., P. P. Fu, T. Cairns and S. K. Yang (1981) Long-range coupling constants for structural analysis of complex polycyclic aromatic hydrocarbons by high-field proton magnetic resonance spectroscopy. *Anal. Chem.* **53**, 558–560.
27. Evans, F. E. and R. A. Levine (1986) Conformation and configuration at the central amine nitrogen of a nucleotide adduct of the carcinogen 2-(acetylamino)flourene as studied by ^{13}C and ^{15}N NMR spectroscopy. *J. Biomol. Struct. Dyn.* **3**, 923–934.
28. Christie, R. M. (2000) *Colour Chemistry*, 205 p. Royal Society of Chemistry, Cambridge, UK.
29. Sahu, M., M. B. Saha and S. C. Bera (1995) Azo-hydrazo tautomerization and cis-trans isomerization of 2-carboxy-2′-hydroxy-4′-methyl azobenzene. *J. Photochem. Photobiol. A: Chem.* **89**, 19–24.
30. Joshi, H., F. S. Kamounah, C. Gooijer, G. van der Zwan and L. Antonov (2002) Excited state intramolecular proton transfer in some tautomeric azo dyes and Schiff bases containing an intramolecular hydrogen bond. *J. Photochem. Photobiol. A: Chem.* **152**, 183–191.
31. Timko, A. L., C. H. Miller, F. B. Johnson and E. Ross (2001) In vitro quantitative chemical analysis of tattoo pigments. *Arch. Dermatol.* **137**, 143–147.
32. Wilkes, T. D. I. (1986) The complications of dermal tattooing. *Ophthalmic Plast. Reconstr. Surg.* **2**, 1–6.
33. Long, G. E. and L. S. Rickman (1994) Infectious complications of tattoos. *Clin. Infect. Dis.* **18**, 610–619.
34. Satchithananda, D. K., J. Walsh and P. M. Schofield (2001) Bacterial endocarditis following repeated tattooing. *Heart* **85**, 11–12.
35. Chunlertrith, K., W. Sukeepaisarnjaroen, P. Mairiang, Y. Urwijitaroon, K. Takase, T. Yamauchi, H. Yoshimura and Y. Tameda (2000) Clinico-epidemiology of hepatitis C viral infection in northeastern Thailand. *Southeast Asian J. Trop. Med. Public Health* **31**, 273–276.
36. Ford, P. M., M. Pearson, P. Sankar-Mistry, T. Stevenson, D. Bell and J. Austin (2000) HIV, hepatitis C and risk behaviour in a Canadian medium-security federal penitentiary. Queen's University HIV Prison Study Group. *Queen's J. Med.* **93**, 113–119.
37. Silverman, A. L., J. S. Sekhon, S. J. Saginaw, D. Wiedbrauk, M. Balasubramaniam and S. C. Gordon (2000) Tattoo application is not associated with an increased risk for chronic viral hepatitis. *Am. J. Gastroenterol.* **95**, 1312–1315.
38. Haley, R. W. and R. P. Fischer (2001) Commercial tattooing as a potentially important source of hepatitis C infection. Clinical epidemiology of 626 consecutive patients unaware of their hepatitis C serologic status. *Medicine* **80**, 134–151.
39. Post, J. J., K. A. Dolan, L. R. Whybin, I. W. Carter, P. S. Haber and A. R. Lloyd (2001) Acute hepatitis C virus infection in an Australian prison inmate: tattooing as a possible transmission route. *Med. J. Aust.* **174**, 183–184.
40. Braithwaite, R. L., T. Stephens, C. Sterk and K. Braithwaite (1999) Risks associated with tattooing and body piercing. *J. Public Health Policy* **20**, 459–470.
41. Center for Disease Control and Prevention (2004) Can I get HIV from getting a tattoo or through body piercing? Available at: http://www.cdc.gov/hiv/pubs/faq/faq27.htm. Accessed 1 June 2004.
42. Tazelaar, D. J. (1970) Hypersensitivity to chromium in a light-blue tattoo. *Dermatologica* **141**, 282–287.
43. Rubegni, P., M. Fimiani, G. de Aloe and L. Andreassi (2000) Lichenoid reaction to temporary tattoos. *Contact Dermatitis* **42**, 117–118.
44. Zirkin, H. J., I. Avinoach and P. Edelwitz (2001) A tattoo and localized lymphadenopathy: a case report. *Cutis* **67**, 471–472.
45. Mahalingam, M., E. Kim and J. Bhawan (2002) Morphea-like tattoo reaction. *Am. J. Dermatopathol.* **24**, 392–395.
46. Goldstein, N. (1967) Mercury-cadmium sensitivity in tattoos. A photoallergic reaction in red pigments. *Ann. Intern. Med.* **67**, 984–989.
47. Nguyen, L. Q. and H. B. Allen (1979) Reactions to manganese and cadmium in tattoos. *Cutis* **23**, 71–72.
48. Verdich, J. (1981) Granulomatous reaction in a red tattoo. *Acta Dermato-Venereol.* **61**, 176–177.
49. Bendsoe, N., C. Hansson and O. Sterner (1991) Inflammatory reactions from organic pigments in red tattoos. *Acta Dermato-Venereol.* **71**, 70–73.
50. Duke, D., S. S. Urioste, J. S. Dover and R. R. Anderson (1998) A reaction to a red lip cosmetic tattoo. *J. Am. Acad. Dermatol.* **39**, 488–490.
51. Juhlin, L. and S. Ohman (1968) Allergic reactions to mercury in red tattoos and in mucosa adjacent to analgam. *Acta Dermato-Venereol.* **48**, 103–105.
52. Fields, J. P., W. D. Little Jr. and P. E. Watson (1968) Discoid lupus erythematosus in red tattoos. *Arch. Dermatol.* **98**, 667–669.
53. Taaffe, A., A. G. Knight and R. Marks (1978) Lichenoid tattoo hypersensitivity. *Br. Med. J.* **1**(6113), 616–618.
54. Clarke, J. and M. M. Black (1979) Lichenoid tattoo reactions. *Br. J. Dermatol.* **100**, 451–454.
55. Taaffe, A. and E. H. Wyatt (1980) The red tattoo and lichen planus. *Int. J. Dermatol.* **19**, 394–396.
56. Bagley, M. P., R. A. Schwartz and W. C. Lambert (1987) Hyperplastic reaction developing within a tattoo. Granulomatous tattoo reaction, probably to mercuric sulfide (cinnabar). *Arch. Dermatol.* **123**, 1557–1561.
57. Sowden, J. M., J. P. Byrne, A. G. Smith, C. Hiley, V. Suarez, B. Wagner and D. N. Slater (1991) Red tattoo reactions: X-ray microanalysis and patch-test studies. *Br. J. Dermatol.* **124**, 576–580.
58. Sowden, J. M., P. H. Cartwright, A. G. Smith, C. Hiley and D. N. Slater (1992) Sarcoidosis presenting with a granulomatous reaction confined to red tattoos. *Clin. Exp. Dermatol.* **17**, 446–448.
59. Tresukosol, P., S. Ophaswongse and P. Kullavanijaya (1997) Cutaneous reaction to cosmetic lip tattooing. *Contact Dermatitis* **36**, 176–177.
60. Pauluzzi, P., M. Giordani, G. F. Guarneri and M. Pascone (1998) Chronic eczematous reaction to red tattoo. *J. Eur. Acad. Dermatol. Venereol.* **11**, 187–188.
61. Kirsch, N. (1972) Malignant melanoma developing in a tattoo. *Int. J. Dermatol.* **11**, 16–20.
62. Wolfort, F. C., J. E. Hoopes, H. S. Filtzer and T. C. Cochran (1974) Superficial melanoma in a tattoo. *Br. J. Plast. Surg.* **27**, 303–304.
63. Bashir, A. H. (1976) Basal cell carcinoma in tattoos: report of two cases. *Br. J. Plast. Surg.* **29**, 288–290.
64. Bartal, A. H., Y. Cohen and E. Robinson (1980) Malignant melanoma arising at tattoo sites used for radiotherapy field marking. *Br. J. Radiol.* **56**, 913–914.
65. Earley, M. J. (1983) Basal cell carcinoma arising in tattoos: a clinical report of two cases. *Br. J. Plast. Surg.* **36**, 258–259.

66. Weiner, D. A. and R. K. Scher (1987) Basal cell carcinoma arising in a tattoo. *Cutis* **39**, 125–126.
67. Soroush, V., A. W. Gurevitch and S. K. Peng (1997) Malignant melanoma in a tattoo: case report and review of literature. *Cutis* **59**, 111–112.
68. Sangueza, O. P., S. Yadav, C. R. White Jr. and R. M. Braziel (1992) Evolution of B-cell lymphoma from pseudolymphoma. A multidisciplinary approach using histology, immunohistochemistry, and Southern blot analysis. *Am. J. Dermatopathol.* **14**, 408–413.
69. Kuperman-Beade, M., V. J. Levine and R. Ashinoff (2001) Laser removal of tattoos. *Am. J. Clin. Dermatol.* **2**, 21–25.
70. Hu, X. H., W. A. Wooden, S. J. Vore, M. J. Cariveau, Q. Fang and G. W. Kalmus (2002) In vivo study of intradermal focusing for tattoo removal. *Lasers Med. Surg.* **17**, 154–164.
71. Peach, A. H. S., K. Thomas and J. Kenealy (1999) Colour shift following tattoo removal with Q-switched Nd-YAG laser (1064/532). *Br. J. Plast. Surg.* **52**, 482–487.
72. Ross, E. V., S. Yashar, N. Michaud, R. Fitzpatrick, R. Geronemus, W. D. Tope and R. R. Anderson (2001) Tattoo darkening and nonresponse after laser treatment. *Arch. Dermatol.* **137**, 33–37.
73. Varma, S., N. A. Swanson and K. K. Lee (2002) Tattoo ink darkening of a yellow tattoo after Q-switched laser treatment. *Clin. Exp. Dermatol.* **27**, 461–463.