

After the dyeing procedure was completed, all the monkeys were restrained in ophthalmological chairs, thus preventing the animals from touching the scalp area. Urine samples were collected at 6, 12, and 24 hours and from then on at 24-hour intervals for 7 days. For both species and for each time period, total urine weights were recorded and an aliquot was removed for analysis.

All urine samples were filtered and assayed in para-phenylenediamine (PPD)/Triton/toluene with a liquid scintillation spectrometer. A [¹⁴C]toluene internal standard (100,000 cpm) was added to each counting vial to determine the extent of quenching. The counting cocktail was 81% efficient and the background was 22 cpm. Most specimens were also counted by the wet ashing method (11). The assay values listed in the tables have been corrected for incomplete excretion from internal application. For the latter, see Table 30.1.

Samples of hair or of the horny layer were digested overnight in counting vials, each containing 1 ml of Unisol. The digested samples were decolorized by the addition of 50% hydrogen peroxide and each was diluted with 15 ml of Unisol complement. Clear samples were equilibrated in the counting chamber at 4°C before counting on a Packard Tricarb liquid scintillation spectrometer. Three samples were analyzed for each hair lot and one of the stratum corneum, with three radioactivity determinations for each sample.

Two methodological approaches (denoted “Application Only” and “Application and Wear”), reflecting different experimental objectives, have been developed in the course of this study. In the first one, the hair was removed immediately following the completion of the coloring procedure; in the other, the hair was left on. The first approach allowed us to evaluate the extent of skin penetration by hair dyes resulting from the hair coloring procedure alone. The second approach recognized that (a) people color their hair to wear it as such, and (b) the dyed hair represents a reservoir of dye moieties that, through a variety of routes, may become bioavailable.

The data on total excretion of radiolabeled dye ingredients are given in Table 30.2. In most cases, they reflect the counts obtained over 144 hours following application of the dye; occasionally a time span of 96 hours was used if the counts at longer times were at the background level.

Two entries of Table 30.2, namely (T_{1/2}) and total dose excretion, should be clarified. It was found that the urinary excretion followed satisfactory first-order kinetics and thus, time required for 50% excretion (T_{1/2}) was employed as an additional quantifying parameter. Regarding the dose, bear in mind that in the process of hair coloring, the product is usually applied in the form of a viscous lotion and uniformly distributed within the hair mass. Clearly, only the product that is in contact with the scalp serves as a dye reservoir available for skin penetration. The quantity available depends on the

TABLE 30.2
Parameters of Percutaneous Absorption of Hair Dyes

Hair Color Category	Labeled Ingredient	Species	Number of Subjects	Total Dose Excretion [% (#SD)]	T _{1/2} of Urinary Excretion (hr)
Permanent (oxidative)	2,4-Diaminoanisole (DAA)	Man	3	0.022 (0.01)	18
		Rhesus monkey	2	0.032	20
	Resorcinol	Man	3	0.076 (0.03)	31
		Rhesus monkey	3	0.177 (0.03)	31
	4-Amino-2-hydroxytoluene	Man	3	0.20 (0.10)	24
	p-Phenylenediamine (PPD)	Man	5	0.190 (0.06)	16
Rhesus monkey		3	0.182 (0.06)	22	
Semipermanent	2-Nitro-PPD	Man	3	0.143 (0.04)	24
		Rhesus monkey	3	0.551 (0.10)	24
	4-Amino-2-nitrophenol	Man	3	0.235 (0.08)	10
	HC Blue No. 1	Man	5	0.151 (0.12)	18
Rhesus monkey		3	0.127 (0.03)	40	