U1 may be a conjugate of U3 as U1 disappeared after acidic hydrolysis and reappeared at the time of U3. The metabolite identity of U2 is unknown. NAT2 genotype results found three subjects classified as slow acetylators and five as intermediate acetylators.

Radioactivity suggests that only PPD-related metabolites were present in the urine of human subjects exposed to oxidative hair dye containing [14C]-PPD. There was no evidence of systemic absorption of reaction products of the oxidative hair dye process via high molecular weight. Also, that NAT2 genotype is independent of PPD metabolism after topical application of hair dye containing PPD. The possible association of NAT2 slow acetylator genotype and higher bladder cancer risk from exposure of PPD is not supported by this data.

30.3.2 Study 2

Skare and Nohynek et al. (15) completed an abstract on the overview of *in vitro*, *in vivo*, and clinical studies on the metabolism of oxidative hair dyes. In regards to *in vivo* percutaneous absorption of hair dye in humans, the abstract discusses metabolism studies with *in vivo*, widely used hair dye aromatic amines via the oral or dermal route. Results demonstrate that the aromatic amines are N-acetylated in the skin by NAT1, a detoxification pathway. N-acetylation is also a major hepatic pathway and there is no evidence of hepatic metabolic activation. Hepatic phase II conjugation reactions are also indicated as main pathways for the metabolism of some hair dyes. After hair dye use in human subjects, NAT2 NAT2 genotype does not influence urinary profile of PPD. This is inconsistent with the theory that hair dye may be linked to an increased bladder cancer risk in NAT2 slow acetylators.

30.3.3 Study 3

Subsequent to this study, Gube et al. (2011) investigated whether biological monitoring of aromatic diamines can quantify the occupational exposure of hairdressers to permanent hair dyes. Level were compared to levels in people after recent application of hair dyes (16).

Permanent hair dyes were used. Substances, 2,5-toluylene diamine (2,5-TDA) and p-phenylene diamine (p-PDA) are predominantly the active constituents of permanent hair dyes.

All operations associated with potential exposure to hair dyes were documented, including mixing color, applying color, washing hair after dyeing, and cutting freshly colored hair. Participants recorded whether or not they were wearing gloves.

This study involved 52 hairdressers, aged 16–63 years, 40 female and 12 male, from 16 salons. A control group of 19 people from the general population, aged 22–64 years old, 10 males and 9 females, was also included. All included participants stated no use of personal hair dyes 4 days prior to the study.

Urine samples were at the following times: the morning before the first shift of the work week, pre- and post-shift at the third day of the week, and pre- and post-final day of the work week. Hairdressers were asked to wash hands before handling urine samples to avoid external contamination. For the control group, spot urine samples were taken. Urine samples were frozen at −18°C immediately after collection; if this was not possible these were stored at 4–8°C overnight.

Gas chromatography-mass spectrometry (GC/MS) was used for determination of 2,5-TDA and p-PDA. Mean accuracy for 2,5-TDA was 97.3% and for p-PDA it was 93.8%. A 96-well plate photometer was used to determine urinary creatinine concentrations. Internal quality controls and biannual participation in round robins ensured quality of the urinary creatinine assay. Corrections for creatinine were always done for concentrations of urinary diamines.

For statistical analysis, calculations were done to obtain the medians of all individual values of each participant of both 2,5-TDA and p-PDA and the 95th percentile. An analysis of variance was conducted to detect whether there was an intra-shift effect, an effect across a working week,