buffer in the diffusion cell receptor fluid. Various receptor fluids with different solubility properties have been investigated to increase the partitioning of lipophilic chemicals into the receptor fluid that have penetrated skin during *in vitro* absorption studies. Increasing the lipophilicity of receptor fluids used in *in vitro* absorption studies with chemicals such as Volpo 20, ethanol, methanol, BSA, or cyclomethicone can increase the partitioning of a lipophilic chemical from skin into the receptor fluid (5–7). In an *in vitro* percutaneous absorption study of azo pigment 1-[4-phenylazophenylazo]-2-naphthol (PAN) (the color component of the certified color D&C Red No. 17), low absorption of PAN occurred in human and porcine skin exposed to consumer products containing this certified color additive (8). For the suntan product studied, most of the applied PAN was washed off the surface of the skin as unpenetrated color. The majority of PAN that penetrated remained in the skin after 24 hours, and typically a very small amount (0.02% to 0.33% of the applied dose) was found in the receptor fluid after 24 hours for both human cadaver and porcine skin. Extended absorption studies (72 hours) showed that PAN, which penetrated skin within 24 hours, remained in the skin, with little PAN diffusing out into the receptor fluid over 72 hours. These results suggest that little of the PAN remaining in skin after 24 hours is available for systemic absorption. Since there were high levels of PAN found remaining in skin after 24 and 72 hours in human skin—12.6% and 15.3% of the applied dose, respectively—an investigation was conducted testing different lipophilic receptor fluids to determine if they increased the partitioning of PAN out of the skin into the receptor fluid. Four lipophilic receptor fluids were tested in addition to HHBSS with 4% BSA, which included 1%, 3%, and 6% Volpo 20 as weight % in deionized water and ethanol:water (50:50). These studies were conducted using human cadaver skin (8). The solubility of PAN in 6% Volpo 20 (0.26 mg/mL) was approximately 37% higher than in HHBSS + 4% BSA (0.19 mg/mL). However, no significant differences were observed in the penetration and subsequent partitioning of PAN from the skin into the two receptor fluids. The lowest solubility of PAN was observed in the ethanol:water (50:50; 0.04 mg/mL) receptor fluid, and yet absorption was found to significantly increase when this receptor fluid was used in the penetration studies. However, care must be taken not to damage the barrier integrity of skin with the receptor fluid selected for an *in vitro* study. It has been shown that a methanol:water (50:50) solution caused damage to rat skin during an *in vitro* absorption study (5). Therefore, the likely reason for the enhanced absorption of PAN with the ethanol:water (50:50) receptor fluid in the skin penetration study is probably due to skin damage.

It is preferable to use a physiological buffer to maintain viability of skin even when metabolism is not measured to simulate *in vivo* conditions. The viability of skin can be maintained by using a balanced salt solution or a tissue culture medium. Addition of 4% BSA may be enough in some cases to enhance receptor fluid levels of applied compound without altering skin viability (9).

28.3 SYSTEMIC ABSORPTION

In *in vitro* skin absorption/penetration studies, definitions of absorption and penetration must be clearly defined. Skin/dermal/percutaneous *absorption* represents the amount of topically applied chemical that is ultimately determined to be systemically available. This usually constitutes receptor fluid contents at the end of an *in vitro* study. However, if a substantial amount of test compound remains in the skin (skin reservoir), *absorption* would constitute receptor fluid content plus skin content if it is determined that material remaining in the skin ultimately diffuses and partitions into the receptor fluid. Therefore, it must be determined for an individual study whether the stratum corneum and/or viable epidermal/dermal content should be considered systemically available. Skin/dermal/percutaneous *penetration* represents the total amount of topically applied chemical that is found in the receptor fluid plus the skin at the end of a study. However, not all of this material may be systemically available for absorption.

The guideline for skin absorption studies recommended by the European Union's Scientific Committee for Consumer Products (SCCP) requires that material remaining in the viable skin levels (exclusive of stratum corneum) be considered as systemically absorbed (10). The Organization for