for 24 hours in flow-through diffusion cells. However, a limitation of using the MTT assay is that it is a terminal assay and can only be conducted at the end of a study when the skin disc is removed from the diffusion cell. As previously noted, glucose utilization monitored by lactate formation and measurement can be conducted throughout the course of an experiment.

19.3 SKIN ENZYME LEVELS

Absolute levels of skin metabolic capacity often differ from liver metabolic capacity for specific enzymes and differ across species. Since many different animal species and in vitro skin models are currently being used for penetration/absorption studies, it is important to understand the differences when attempting to promote the use of a specific model as a replacement for intact human skin for dermal safety assessments. There are four detailed reviews pertaining to this subject that compare species and model differences in skin metabolic conversion capacity (1, 12–14). The cytochrome P450 activity in skin is only a small fraction of liver activity. This is true for pig, guinea pig, mouse, rat, and human skin. Therefore, there is less chance for reactive metabolite formation due to cytochromes P450 activity in skin when compared to other non–cytochrome P450 hydrolytic activity and phase II metabolic levels.

When comparing in vitro human skin models, cytochrome P450 activity is still low compared to liver activity (1). The in vitro human skin models are generally similar in cytochrome P450 activity compared with animal skin models. The activity of NADH/NADPH quinone reductase in intact human skin is similar to that of primary human keratinocytes, EpiDerm and EpiSkin. When comparing esterase activity between species, human and pig skin have similar esterase activity, while rat skin has lower esterase activity (1). Other early work found that diethyl malonate was metabolized during skin absorption using pig skin mounted in a penetration cell (15). The almost total metabolism of diethyl malonate was determined to be mainly due to esterases in the skin. Heat treatment of the skin to destroy esterase activity resulted in much more of the parent compound being absorbed unmetabolized through skin.

Comparing phase II conjugating enzyme activity across animal species is difficult due to a lack of data. However, comparison can be made across in vitro human skin models. Phase II conjugating activity is similar in human skin, primary human keratinocytes, and the HaCaT cell line. The activity of glutathione S-transferase was found to be either lower or higher in EpiDerm, EpiSkin, and SkinEthik than in intact human skin but was within tenfold for glutathione S-transferase activity across whole skin and the in vitro human skin models (1).

N-acetyltransferase activity in EpiDerm, EpiSkin, and SkinEthik are either lower or higher than the activity of intact human skin but are generally within twofold to threefold activity when compared with intact human skin (1). N-acetyltransferase activity in whole skin, measured by gene expression levels, is an enzyme where there is many-fold higher activity in skin compared with liver (16).

The use of animal skin or in vitro human skin models (e.g., cell lines or reconstructed models) should have enzymatic activity like that of intact human skin to be a good predictive model for chemical skin absorption. It is important to be cognizant of enzyme activity differences in these animals and models when choosing to use a specific model. Specific chemical toxicity after dermal contact is dependent upon the extent of skin absorption, which is a function of (1) chemical-specific physiochemical properties that influence diffusion properties and (2) the extent of chemical metabolism while diffusing through the skin layers.

19.4 EXAMPLES OF SPECIFIC CHEMICAL SKIN ABSORPTION AND METABOLISM STUDIES

The following skin absorption and metabolism studies were selected as examples to illustrate the types of compounds that are metabolized in skin upon penetration/absorption. In many cases, the metabolites formed from the parent compounds have been identified, and important metabolic reactions in skin have been elucidated.