COMPARATIVE STUDIES ON THE EFFECTS OF WATER, ETHANOL AND WATER/ETHANOL MIXTURES ON CHEMICAL PARTITIONING INTO PORCINE STRATUM CORNEUM AND SILASTIC MEMBRANE

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ABSTRACT

The effects of water and ethanol vehicles on stratum corneum and silastic membrane partitioning of 11 industrial and agricultural compounds were studied to aid in characterizing and assessing risk from skin exposure. Zero percent, 50% and 100% aqueous ethanol solutions were used as solvents for ^{14}C labeled phenol, 4-nitrophenol, pentachlorophenol, dimethyl parathion, parathion, chloropyrifos, fenthion, triazine, atrazine, simazine and propazine. Compound partitioning between the solvents and porcine stratum corneum/silastic membrane were estimated. Stratum corneum was exposed to aqueous ethanol ranging from 0% to 100% v/v ethanol in 20% increments and Fourier transform infrared spectroscopy (FT-IR) was used to obtain an index of lipid disorder. Gravimetry and FT-IR were used to demonstrate lipid extraction in aqueous ethanol solutions. Partitioning patterns in silastic membranes resembled those in stratum corneum and were correlated with octanol/water partitioning. Partitioning was highest in water and was higher from 50% ethanol than from 100% ethanol, except for parathion, 4-nitrophenol, atrazine and propazine. Correlation existed between molecular weight and partitioning in water, but not in ethanol and ethanol/water mixtures. Lipid order, as reflected in FT-IR spectra, was not altered. These studies suggest that stratum corneum partitioning of the compounds tested is primarily determined by relative compound solubility between the stratum corneum lipids and the donor solvent. Linear relationships existed between octanol/water partitioning and stratum corneum partitioning. Partitioning was also correlated with molecular weight in water solvent systems, but not in ethanol and ethanol/water mixtures. Ethanol and ethanol/water mixtures altered the stratum corneum through lipid extraction, rather than through disruption of lipid order.

Keywords: Dermal absorption; Partitioning; Chemical mixtures

INTRODUCTION

Skin exposure to potentially toxic industrial and agricultural chemicals is a common occurrence. Predicting the rate at which chemicals move across skin is important for the assessment of risk associated with skin exposure to these compounds. Most toxicological risk assessment studies assess dermal absorption after neat chemical exposure, yet environmental and occupational exposures are to chemicals in solvents.

Estimating skin permeability depends on adequate description and understanding of the processes that influence the barrier properties of skin. The stratum corneum is the primary barrier to exogenous chemical absorption and water loss through mammalian skin. It consists of layers of tightly packed, flattened, keratin-enriched, anucleate corneocytes, embedded in an intercellular lipid matrix (Bouwstra et al 2003). The main constituents of the lipid matrix are long chain ceramides, fatty acids, cholesterol and triglycerides (Monteiro-Riviere et al 2001). These lipids form long lamellae parallel to the corneocyte surfaces. Inside the lamellae, at physiological temperatures, the lipids are arranged in bilayers consisting of ordered, crystalline phases on both sides of a narrow, central band of fluid lipids (Monteiro-Riviere 1986; Bouwstra et al 2003). Partitioning into corneocytes is dependent on the lipophilicity of the compound. More hydrophilic compounds tend to partition into the corneocytes proteins, while more lipophilic compounds tend to partition into the stratum corneum lipids (Raykar et al 1988). The intercellular lipid matrix is therefore the main route for the passage of lipophilic exogenous chemicals through intact stratum corneum (Albery & Hadgraft 1979).

The rate of chemical absorption through the skin is primarily determined by two factors: partitioning into the stratum corneum and its resistance to diffusion (Scheuplein & Blank 1971). If Fickian diffusion is assumed, permeability should be proportional to diffusivity and partitioning into the stratum corneum lipids. Also, if diffusivity and partitioning can be predicted, it follows that permeability should be predictable. The prediction of partitioning and diffusivity is, however, problematic. The complexity of skin structure, variability in skin lipid composition and thickness, changes in skin induced by solvents and permeants and the effects of metabolizing enzymes on permeants with the possible occurrence of finite capacity processes, makes it unlikely that skin permeability can be accurately predicted based only on permeant and solvent physical–chemical data. Added complexity due to the practically limitless solvent systems that can be encountered causes prediction of

skin permeability and its use in risk assessment to be laden with uncertainty. That said, the use of large sets of empirical data does offer the potential for identifying characteristics of permeants and solvent systems that show consistent effects across a wide range of experimental conditions. This approach has been used with some success to predict skin permeability in relatively simple systems based on quantifiable molecular characteristics, such as molecular size (weight and volume), octanol/water partition coefficients, H-bonding capacity and electric charge (Potts & Guy 1992). These physico-chemical parameters are predictive of absorption due to their influence on partitioning and diffusivity. However, solvents may change the partitioning and diffusion behavior of compounds in stratum corneum depending on the physico-chemical properties of the solvent and solvent effects on the stratum corneum (Raykar et al 1988; Kai et al 1990; Rosado et al 2003). Solvent induced changes in the stratum corneum could also change its resistance to diffusion. Empirical characterization of the effects of commonly encountered solvents on the stratum corneum could improve the prediction of skin absorption in many real-world scenarios.

Water and ethanol are commonly used solvents in the chemical and pharmaceutical industries. Increased permeant flux has been demonstrated using a variety of permeants, methods and skin models using aqueous ethanol solvents in the 40–70% (v/v) range, compared to higher and lower ethanol concentrations (Berner et al 1989; Kurihara-Bergstrom et al 1990; Megrab et al 1995; Kim et al 1996; Levang et al 1999; Panchagnula et al 2001). The suggested mechanisms by which ethanol affects stratum corneum permeability include lipid extraction, increased lipid fluidity, effects on the putative pore pathway, enhanced drug solubility in stratum corneum lipids, changes in stratum corneum hydration, altered keratinized protein and permeant-ethanol copermeation. Pretreatment of human stratum corneum to extract lipids have been demonstrated to influence stratum corneum partitioning of lipophilic compounds from an aqueous donor solution (Raykar et al 1988). The effects of ethanol pretreatment on the barrier properties of hairless mouse skin to nicotinamide in an aqueous solution indicated that lipid extraction compromised the stratum corneum barrier (Kai et al 1990). This study builds on previous work investigating chemical partitioning into the stratum corneum from water, ethanol and water/ethanol mixtures by extending the range of model compounds to those with toxicological significance and by determining the effects on stratum corneum/solvent partitioning using ethanol, water and ethanol/water

mixtures as donor solvents, not as pretreatments. Partitioning patterns and solvent effects on stratum corneum lipid order and lipid extraction were used to illuminate the processes that determine the rate of skin absorption.

MATERIALS AND METHODS

Chemicals

CCl_4 and C-14 radio labeled phenol, 4-nitrophenol, pentachlorophenol, dimethyl parathion, parathion, atrazine, and simazine were obtained from Sigma (St. Louis, MO). Chlorpyrifos, fenthion, triazine (1,3,5-triethylhexahydro-1,3,5-triazine; also called 1,3,5-triethylhexahydro-s-triazine) and propazine were obtained from American Radiolabeled Chemicals (St. Louis, MO). The purity ranged from 95% to 99.5% and the radioactivity ranged from 9 to 76.6 mCi/mmol. All C-14 radio labels were situated in the ring structure of the molecules. Pure ethanol was obtained from Aaper Alcohol and Chemical Co. (Shelbville, KY).

Stratum corneum/vehicle and silastic membrane/vehicle partition coefficient determination

Stratum corneum/vehicle and silastic membrane/vehicle partition coefficients were estimated according to methods previously described (Baynes 2000). In short, stratum corneum and epidermis layers were removed from abdominal skin of female weanling Yorkshire pigs after heat treatment and then immersed in 0.25% trypsin (Sigma Chemical Co., St. Louis, MO) for 24 h. The stratum corneum was then dried in a Fisherbrand Dessicator Cabinet (Fisher Scientific, Pittsburgh, PA) with Drierite™ anhydrous calcium sulfate (WA Hammond Drierite Company, Xenia, Ohio), weighed (5–8 mg per sample) using a Mettler AE 200 scale (Mettler Toledo, Columbus, OH) and placed in vials. Three ml of the solvents with 100 μg radio labeled compound was added to the stratum corneum sample vial (n=5), and capped. After 24 h, the stratum corneum sample was removed and gently blotted on Kimwipe™ to remove excess solution. Two hundred and fifty μl of the vehicle was removed, by pipetting from the center of the fluid mass, for direct counts using Ecolume (ICN Costa Mesa, CA). For determination of radio labeled compound in the stratum corneum, stratum corneum samples were combusted in a Packard Model 306 Tissue Oxidizer (Packard Chemical Co., Downers Grove, IL) and then analyzed using a Packard Model 1900TR Liquid Scintillation Counter (Packard Chemical Co., Downers Grove, IL). The same method was used to estimate silastic membrane/vehicle partitioning. Biomedical

grade silastic membrane was obtained from Dow Corning Corporation (Hemlock, MI). The research adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985).

FT-IR

FT-IR transmission spectra were obtained using a Perkin–Elmer Spectrum 1000 FT-IR spectrometer (Perkin–Elmer Inc., Wellesley, MA). Dried stratum corneum was prepared as for stratum corneum/vehicle partition coefficient determination. Circular stratum corneum samples (c. 32 mm diameter) were cut from a single sheet of stratum corneum for each experiment (n=3). The stratum corneum samples were immersed in 10 ml of solvent for 12 h in closed 20 ml vials. The solvents used were pure water, aqueous ethanol in 10% increments of ethanol v/v and pure ethanol. Treated samples were gently blotted on Kimwipe™ to remove excess solution. Samples were then suspended in a demountable FT-IR liquid cell (Pike Technologies, Madison, WI) for determination of transmission spectra. Samples were then exposed to a desiccating atmosphere at room temperature for 24 h and the transmission spectra of the dried samples were determined.

Lipid extraction

Dried stratum corneum was prepared as for stratum corneum/vehicle partition coefficient determination. Stratum corneum samples (n=4), within a weight range of 40–60 mg, were weighed at the start of the procedure using a Mettler AE 200 scale (Mettler Toledo, Columbus, OH) and placed in closed vials with 10 ml water, 50% aqueous ethanol (v/v) or 100% ethanol. The stratum corneum samples were removed after 24 h, gently blotted on Kimwipe™. The samples were then placed in a desiccating atmosphere for 24 h and weighed again. The solvents were evaporated at 50 °C using nitrogen gas in a Zymark TurboVap evaporator (Zymark Corporation, Hopkinton, MA) and the precipitate was redissolved in 0.5 ml CCl_4. A thin film of precipitate was prepared by spreading a drop of the redissolved precipitate solution on the surface of a KBr crystal (Pike Technologies, Madison, WI) and allowing the CCl_4 to evaporate at room temperature. FT-IR transmission spectra were obtained using a Perkin–Elmer Spectrum 1000 FT-IR spectrometer (Perkin–Elmer Inc., Wellesley, MA).

Data processing and statistical analysis

For partition coefficient determinations, radioactivity content in the vehicle mixture and stratum corneum were normalized to 1000 mg vehicle ($C_{vehicle}$) and 1000 mg stratum corneum ($C_{stratum\ corneum}$), respectively. The log stratum corneum/vehicle partition coefficient was determined from the equation: $\log PC = \log C_{stratum\ corneum}/C_{vehicle}$. Standard errors were determined for all data sets. Log *K* octanol/water values were obtained from the literature (Howard & Meylan 1997), except for triazine. No published log*K* octanol/water values for triazine were available. The SPARC On-Line Calculator (2003 version; available at: http://ibmlc2.chem.uga.edu/sparc/) was used to calculate an estimated log*K* octanol/water for triazine.

FT-IR peaks at 2955 cm^{-1} were used to indicate absorbance due to asymmetric vibration associated with CH_3 functional groups. Peaks around 2854 cm^{-1} indicated CH_2 symmetric vibration and 2925 cm^{-1} indicated CH_2 asymmetric vibration (Parker 1983). "Blue-shift" of these peaks to higher wave numbers were used to indicate decreased lipid order. Peaks around 1740 cm^{-1} indicated absorbance due to C=O bonds associated with COOH functional groups (Raykar et al 1988).

RESULTS

Partitioning

Partitioning into the stratum corneum was highest across all compounds when water was used as a solvent (Figure 1; Table 1). Partitioning from 50% ethanol was higher than from 100% ethanol, except for 4-nitrophenol, parathion, atrazine and propazine, which did not show any significant difference between 50% and 100% ethanol. Stratum corneum partitioning was correlated with octanol/water partitioning (Figure 2). Divergence between octanol/water partitioning and stratum corneum/solvent partitioning became wider in 50% and 100% ethanol as the octanol/water partitioning coefficient increased. The decreased slope of the regression line in 50% and 100% ethanol illustrates this effect. Significant correlation was observed between molecular weight and stratum corneum partitioning when water was used as a solvent (Figure 3), but correlation was poor when 50% or 100% ethanol was used.

Lipid order and extraction

FT-IR did not reveal consistent, significant V_aCH_2 or V_sCH_2 peak shifting (Figure 4). Gravimetry revealed increasing loss of stratum corneum mass after treatment with 50% and 100% aqueous ethanol solutions and no significant change in mass after treatment with water (Figure 5). The FT-IR spectra, obtained from the precipitates of the extracts, indicated that the extracted material predominantly consisted of the lipid fraction of the stratum corneum (Figure 6). Cellular debri were observed in the extracts using light microscopy, indicating that some of the loss in mass was caused by loss of material other than lipids. Infrared absorption in the 1740 cm^{-1} region, associated with COOH groups, was lowered in a concentration dependent manner Figure 7). It is consistent with loss of lipid-associated COOH groups. Dried aqueous ethanol treated samples also revealed concentration dependent lowering of the V_aCH_2 and V_sCH_2 peaks, which also is consistent with loss of lipids from the stratum corneum (Figure 8). Water did not extract observable amounts of lipid.

The presence of ethanol in stratum corneum was detected through its influence on infrared absorption in the 2955 cm^{-1} region associated with CH_3 groups (Figure 8). The enhanced CH_3 associated absorption was absent after drying the samples for 24 h, indicating that ethanol was not present in the samples in significant quantities after 24 h.

DISCUSSION

Dermal absorption and stratum corneum partitioning studies from water are traditionally used to predict risk from skin exposure. Many exposures, however, are from chemical mixtures rather than from pure water. Investigations of the effects of mixed solvents on processes that determine absorption are therefore relevant to the appropriate use of data from studies using aqueous solvents analyzing risk from mixture exposure.

Triazine (1,3,5-triethylhexahydro-1,3,5-triazine; CAS# 7779-27-3) was used as an industrial biocide to control bacteria and fungi in adhesives, fuels, oil storage tanks, cutting fluids, paints, slurries, rubber products and industrial processing chemicals. Although the product had been registered as a biocide since 1967, Data-Call-Inn notices were issued in 1987 and 1992. Reregistration is dependent on additional toxicity and ecological effects data (United States Environmental Protection Agency 1997). Information on triazine's physical

properties is largely absent from the available scientific literature. The log*K* octanol/water value used in this study should be interpreted with caution, since the accuracy of the calculation was not be validated experimentally. Studies on the physical properties of this compound are needed to assist in the interpretation of data for risk analysis procedures.

Partitioning between a donor solvent and a membrane is a function of the relative solubility of a compound in the donor solution and the membrane. The observed partitioning pattern therefore reflects changes in the stratum corneum that alter its solvation properties relative to the donor solvent. The higher partition coefficients obtained in water, compared to pure ethanol and aqueous ethanol, can be explained by the fact that most of the tested compounds are relatively non-polar with log octanol/water partition coefficient values ranging from 1.46 to 5.12, with the exception of triazine, which is hydrophilic. Ethanol consists of a short two-carbon chain, which confers on it the ability to interact with and dissolve relatively non-polar molecules; and a hydroxyl group, which gives it the ability to interact with relatively polar molecules and to form hydrogen bonds. It therefore has the ability to act as a solvent for organic molecules with a wide range of octanol/water partition coefficients. In contrast, water is not an effective solvent for relatively non-polar organic molecules.

The range of stratum corneum/ethanol partition coefficients was much narrower than the range seen with log stratum corneum/water. The influence of partitioning on absorption rate suggests that the expected range of absorption rates of compounds from ethanol will be narrower than the absorption rates from water. The increasing divergence observed between log octanol/water partitioning coefficient and log stratum corneum/solvent partitioning coefficient in 50% and 100% ethanol as the log octanol/water partitioning coefficient increased is therefore consistent with the hypothesis that log stratum corneum/solvent partitioning coefficient is determined by the relative solubility of the solute in the solvent and in the stratum corneum. Divergence also occurred between pure ethanol and aqueous ethanol, as expected. However, the effect was inconsistent exceptions were *p*-nitrophenol, atrazine, propazine and parathion. The log octanol/water partitioning coefficient range of these compounds are between 1.9 and 4. Compounds outside this range exhibited the expected divergence. It is not clear that the effect is dependent on log octanol/water partitioning coefficient. It may be related to specific interactions between functional groups on the compounds and stratum corneum components. More compounds across a wide range of partitioning behavior should be studied to

determine the consistency of the effect. Pentachlorophenol exhibited a lower partitioning into stratum corneum and silastic from water than predicted from its $logK_{o/w}$ value. The pK_a of pentachlorophenol is 4.7, which causes ionization of a significant proportion of the molecules when in a solvent with a pH close to neutral. Polarization increases water solubility thereby lowering stratum corneum/water partitioning.

The stratum corneum has low water content and stratum corneum lipids are ordered into bilayers. These characteristics contribute to the suitability of FT-IR as a useful technique for studying stratum corneum lipid structure (Ongpipattanakul et al 1994). FTIR spectra of lipid bilayers show typical absorption patterns in the 3000–2800 cm^{-1} region that are related to C–H_2 stretching (Parker 1983). Disruption of lipid bilayer structures leads to identifiable changes in the absorption spectra of infrared light passed through, or reflected off, the stratum corneum (Moore et al 1997). This has been used successfully to study the temperature dependent phase behavior of skin lipids (Krill et al 1992) and the effect of chemical absorption promoters on the lipid bilayer structure (Yokomizo & Sagitani 1996; Jaiswal et al 1999). Shifts to a higher wave number of peaks around 2854 cm^{-1} (CH_2 symmetric vibration) and 2925 cm^{-1} (CH_2 asymmetric vibration) indicates decreased order in a lipid membrane due to changes in the mobility of lipid acyl chains (Vaddi et al 2002). In the present study, high variability was observed between stratum corneum samples. This is due to variability in lipid composition and stratum corneum thickness. However, the pattern of solvent induced change was consistent when individual samples were compared before and after treatment. Ethanol, due to its C–H groups, also absorbs IR energy in the 3000–2800 cm^{-1} region. This may confound estimates of peak change due to changes in lipid order. However, ethanol adds to the FT-IR absorbance in the 2955 cm^{-1} region due to the presence of CH_3 groups (Parker 1983). This effect is easily detectable in samples tested directly after removal from ethanol, while it is absent from samples exposed to the atmosphere for 24 h (Figure 8). It suggests that ethanol did not contribute significantly to FT-IR spectra obtained after 24 h. The expected "blue-shift" of FT-IR absorbance peaks in the 3000–2800 cm^{-1} region due to reduced lipid order after ethanol treatment was not found (Figure 4). This may be explained by lipid extraction or loss of lipid disorder with ethanol evaporation. Additionally, the extracted portion of the stratum corneum, which would show most evidence of disorder, does not contribute to the FT-IR absorption obtained after treatment. This finding agrees with results previously reported in the literature (Kai et al 1990).

The ability of ethanol to extract lipids from the stratum corneum has been described previously (Sugibayashi et al 1992; Levang et al 1999). We demonstrated lipid extraction under the same conditions as those used to determine stratum corneum/solvent partitioning using gravimetry in combination with FT-IR (Figure 5, Figure 6 and Figure 7). Lipid extraction results in lowered IR absorbance due to C–H_2 groups in the 3000–2800 cm^{-1} region as well as lowered absorbance due to COOH in the 1740 cm^{-1} region. Although the conditions under which lipid extraction was demonstrated is unlikely to be encountered in topical drug formulations, it indicates a substantial potential for lipid extraction under conditions of excessive skin exposure to ethanol, which may occur in an occupational exposure scenario. Lipid extraction from the stratum corneum results in a lowered mass of lipids within the membrane and a smaller capacity for accepting lipophilic molecules from the donor solution, thereby contributing to lowered partitioning into the stratum corneum. Lipid extraction could also change the lipid composition and solvation properties of the stratum corneum lipids. Stratum corneum samples were dehydrated through storage in a desiccating atmosphere before estimation of control sample weights. However, the hygroscopic nature of pure ethanol could cause further dehydration of the stratum corneum. Lipid extraction, loss of cellular debri and dehydration therefore contributed to the loss of stratum corneum mass after ethanol treatment. It should also be noted that an exposure time of 24 h is likely to show extreme solvent effects, which may differ from solvent effects associated with shorter exposure times. However, initial investigation did not show significant difference between 12 and 24 h changes in FT-IR spectra. More work on lipid extraction, as previously done with other solvents (Monteiro-Riviere et al 2001), is needed to determine the rate and extent of extraction of different types of lipids.

Significant correlation between molecular weight and partitioning into stratum corneum when water was used as a solvent was observed. This may be explained by the relative lipophilic nature of the compounds tested. It has been theorized that increased molecular volume, associated with increased molecular weight, increases the hydrophobic surface area associated with larger molecules and therefore partitioning into stratum corneum (Potts & Guy 1995). Larger molecules are associated with stronger London dispersion forces than smaller molecules due to larger molecular surfaces available for interaction with neighboring molecules, which accounts for the generally higher lipophilicity of larger molecules (Streitwieser 1992). In water, which is strongly lipophobic, increased molecular weight would therefore explain the increased partitioning into the

relatively lipophilic environment within the stratum corneum lipids as molecular weight increases. The correlation of partitioning with molecular weight is, therefore, a reflection of the influence of molecular weight on lipophilicity and not due to a direct causal relationship between partitioning and molecular weight. This hypothesis is in agreement with previous studies showing that lipophilicity drives partitioning behavior in lipid bilayers (Xiang & Anderson 1994). It is also in agreement with the theorized influence of molecular size on partitioning behavior based on scaled-particle theory (Mitragotri et al 1999), which predicts that more energy is required for large molecules to partition into lipid bilayers. Since the size of large molecules tends to reduce partitioning into lipid bilayers, correlation between molecular weight and partitioning may not be present for compounds outside the molecular weight range of the compounds used in this study. Lower correlation between molecular weight and stratum corneum partitioning when ethanol is used as a solvent supports the previously discussed hypothesis that ethanol is a more universal solvent for organic compounds than water. The influence of molecular weight on lipophilicity therefore does not predict the solubility of organic compounds in ethanol to the extent that it does in water. Correlations of molecular size and partitioning should be interpreted with caution when used in attempts to predict permeability due to the effect of molecular size on diffusivity. Molecular size and shape influence diffusivity independent of compound lipophilicity (Xiang & Anderson 1994; Mitragotri et al 1999).

This study demonstrated the determining influence of solvents on processes that control the rate of cutaneous absorption. The predictive value of octanol/water partitioning for partitioning into stratum corneum from water is dependent on similarities between the relative solvation properties of octanol and water to stratum corneum and water. This has important implications for the use of data obtained from compound behavior in one solvent system, typically water, to predict its behavior in another solvent system. The study also suggested that the use of large data sets to identify consistent solvent influences over the behavior of a wide range of chemical permeants offers a workable approach to reduce uncertainty in the risk assessment of real-world dermal exposure to chemicals in commonly used solvents and solvent mixtures. Additional work to elucidate the mechanisms by which broadly repeatable solvent effects function will increase the utility of this approach in chemical and pharmaceutical development.

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Table 1. Log Koctanol/water, molecular weight and log of partitioning between the stratum corneum (sc)/silastic (si) of triazine (TRI), phenol (PHE), p-nitrophenol (PNP), simazine (SIM), atrazine (ATR), methyl parathion (MPA), propazine (PRO), ethyl parathion (EPA), fenthion (FEN), chlorpyrifos (CPY) and pentachlorophenol (PCP). Partitioning values are followed by the standard error of the mean (SEM) (n = 5).

	TRI	PHE	PNP	SIM	ATR	MPA	PRO	EPA	FEN	CPY	PCP
Log Ko/w	-0.11	1.46	1.91	2.18	2.61	2.86	2.93	3.83	4.09	4.96	5.12
Mol. Weight	171.29	94.113	139.11	201.66	215.69	263.21	229.71	291.26	278.33	350.59	266.34
Log Psc/water	0.892	1.081	1.246	1.570	1.722	1.922	1.969	2.952	3.006	3.784	2.534
SEM	0.034	0.037	0.021	0.025	0.113	0.078	0.087	0.065	0.036	0.031	0.037
Log Psc/ethanol+water	0.549	0.784	0.717	1.276	0.695	1.016	0.801	0.987	1.042	1.644	1.629
SEM	0.049	0.046	0.008	0.023	0.049	0.052	0.100	0.056	0.047	0.031	0.036
Log Psc/ethanol	0.337	0.663	0.741	0.880	0.883	0.840	0.905	1.090	0.800	0.960	1.004
SEM	0.077	0.048	0.059	0.008	0.045	0.054	0.064	0.037	0.058	0.066	0.094
Log Psi/water	-1.746	-0.099	-0.945	0.398	1.281	2.058	1.582	2.027	2.442	2.718	0.091
SEM	0.220	0.043	0.211	0.116	0.028	0.009	0.017	0.035	0.036	0.035	0.069
Log Psi/ethanol+water	-2.383	-0.876	-1.649	-0.993	-0.878	0.035	-0.665	0.524	0.470	1.221	-0.941
SEM	0.241	0.028	0.034	0.089	0.029	0.015	0.020	0.047	0.016	0.008	0.071
Log Psi/ethanol	-1.984	-1.963	-2.110	-1.783	-1.753	-1.865	-1.850	-0.906	-1.681	-1.508	-2.360
SEM	0.028	0.013	0.027	0.036	0.045	0.020	0.021	0.121	0.016	0.016	0.038

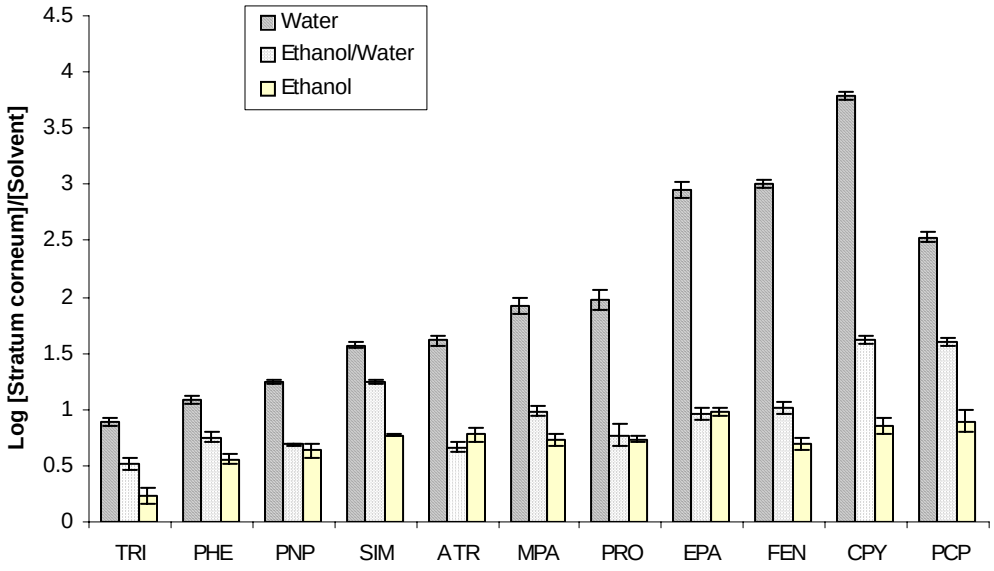

Figure 1. The partitioning between the stratum corneum and the solvent expressed as the log of stratum corneum/solvent concentrations of triazine (TRI), phenol (PHE), p-nitrophenol (PNP), simazine (SIM), atrazine (ATR), methyl parathion (MPA), propazine (PRO), ethyl parathion (EPA), fenthion (FEN), chlorpyrifos (CPY) and pentachlorophenol (PCP) (n = 5).

Plate 1

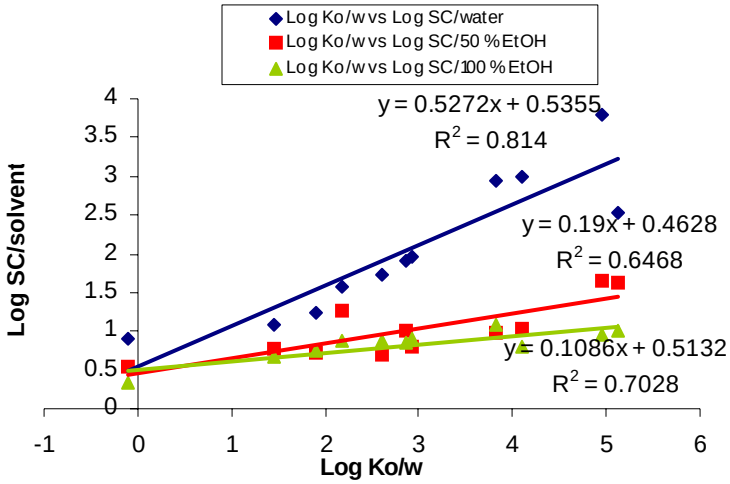

Plate 2

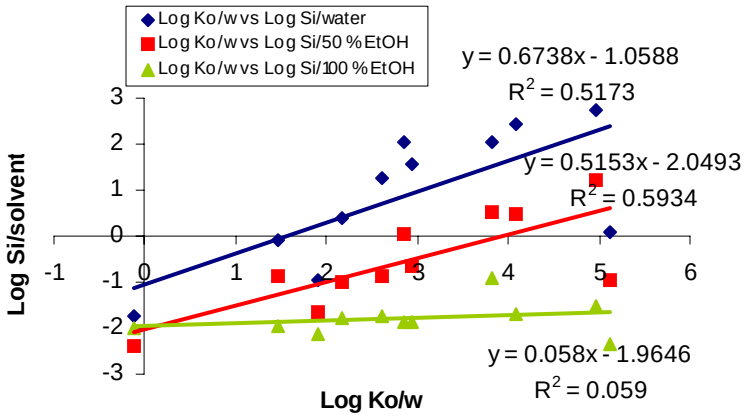

Figure 2 Log Ko/w plotted against the mean log membrane/solvent using stratum corneum (Plate 1) and silastic (Plate 2) as membranes and water, 50 % ethanol and 100 % ethanol as solvents. The linear regression line, regression equation and R^2 value of the plot for each solvent is displayed (n = 5).

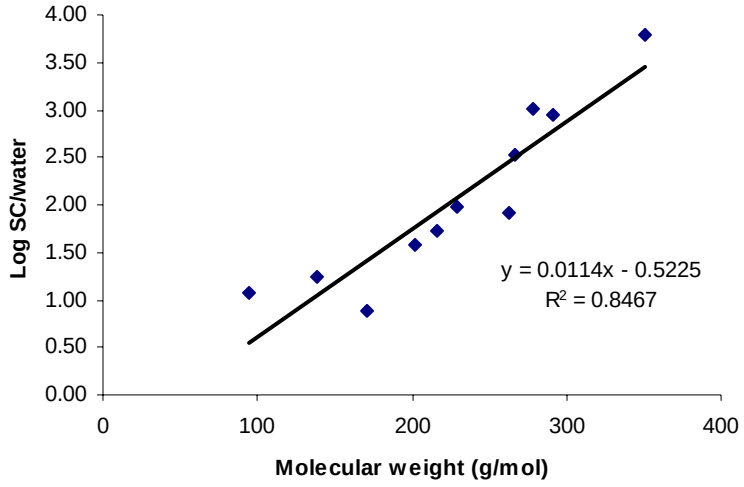

Figure 3. Molecular weight plotted against the mean log SC/water. The linear regression line, regression equation and R^2 value is displayed (n = 5).

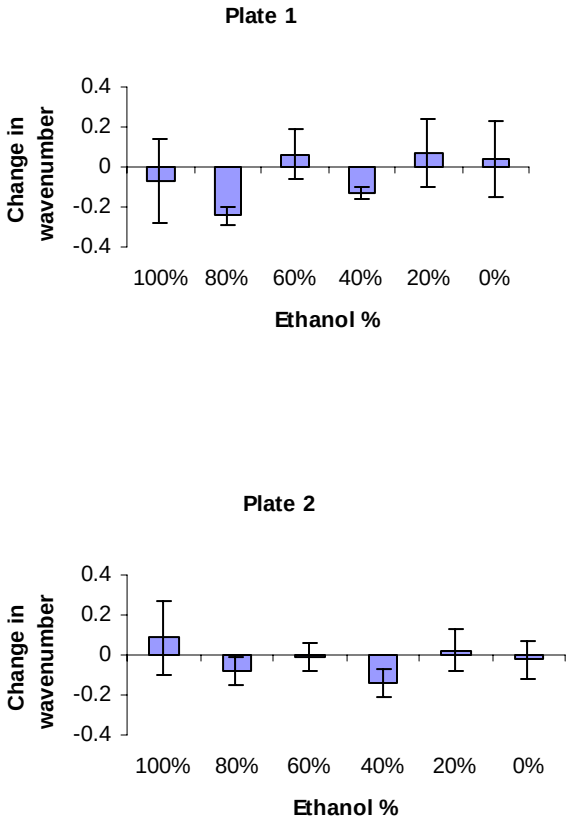

Figure 4. Change in wave number after aqueous ethanol treatment of VaCH2 absorbance in the 2917 cm-1 region (Plate 1) and VsCH2 absorbance in the 2849 cm-1 region (Plate 2) (n=4).

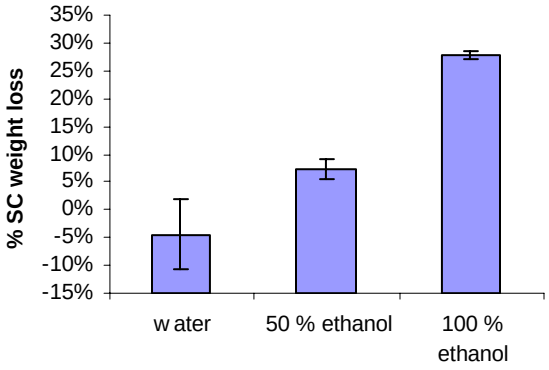

Figure 5. Percentage SC weight loss after 24 hr extraction using water, 50 % ethanol and 100 % ethanol (n = 4).

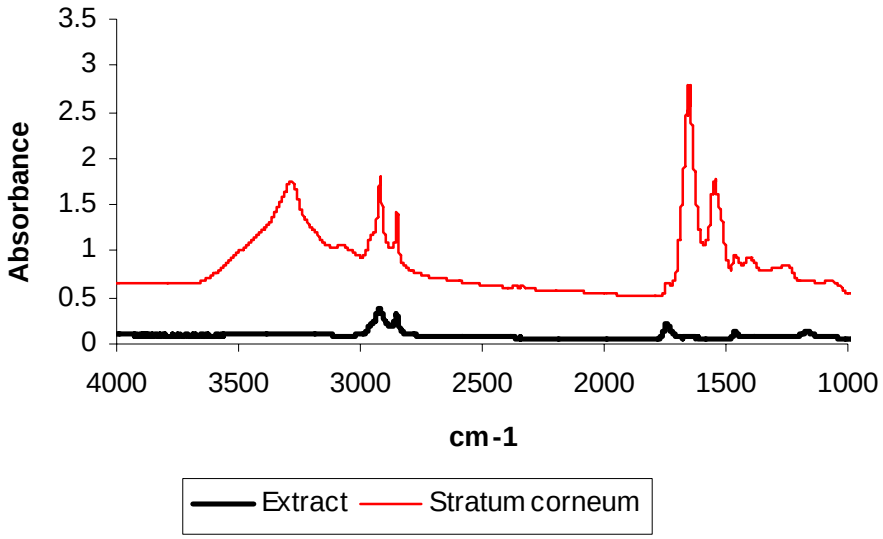

Figure 6. Representative FT-IR absorbance spectra of typical stratum corneum and the precipitate from an ethanol extract of stratum corneum. The peaks between 2800 cm^{-1} and 3000 cm^{-1} are due to IR absorbance at C-H bonds, while the peak at 1743 cm^{-1} is attributed to C=O bonds in carboxyl groups (Parker 1983).

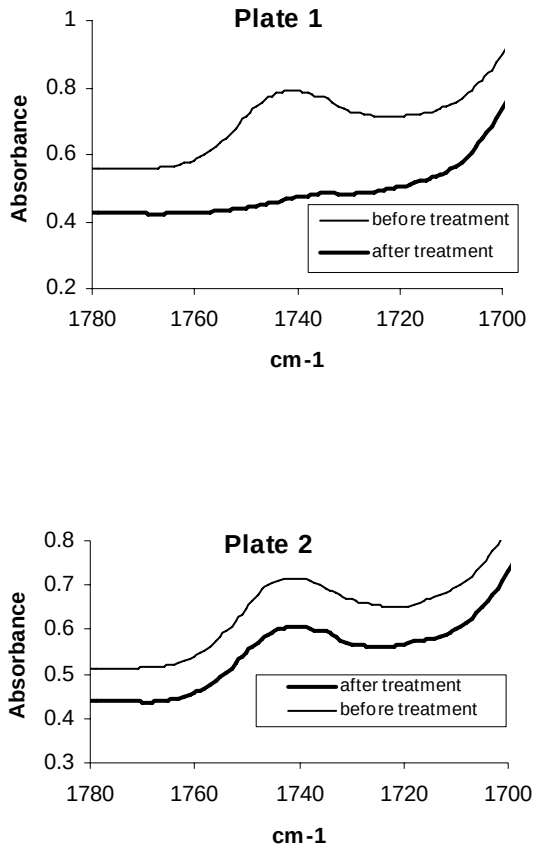

Figure 7. Representative FTIR absorbance spectra of stratum corneum before and after 12 hr exposure to 100 % ethanol (Plate 1) and 10 % ethanol (Plate 2) showing change in absorbance due to COOH in the 1740 cm^{-1} region.

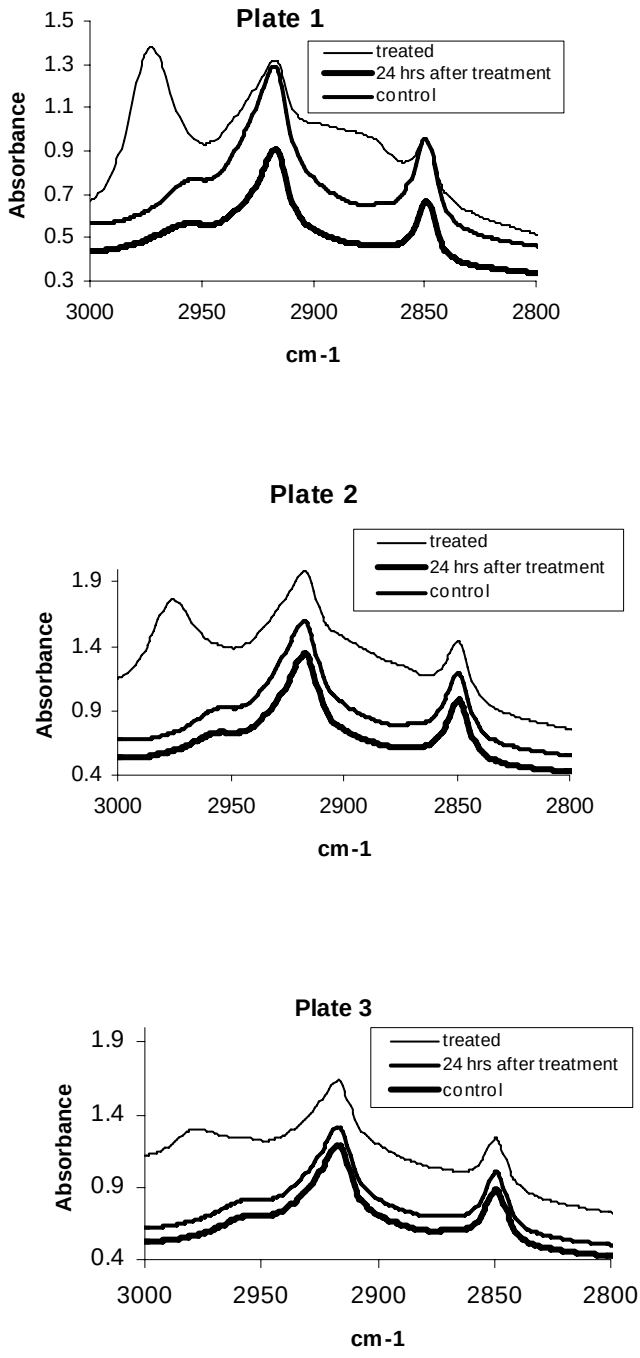

Figure 8. Representative FTIR absorbance spectra of stratum corneum before and after 12 hr exposure to 90 % ethanol (Plate 1), 60 % ethanol (Plate 2) and 30 % ethanol (Plate 3) and again after 24 hours in a dessicating atmosphere, showing change in Va(CH_3) absorbance in the 2955 cm^{-1} region.