Review of dermal effects and uptake of petroleum hydrocarbons

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ABSTRACT

This report serves as an update to and an extension of a previous CONCAWE report on dermal absorption of petroleum hydrocarbons (Petroleum hydrocarbons: their absorption through and effects on the skin, CONCAWE Report 84/54, 1984).

To contribute to health risk assessments associated with dermal exposure, this report evaluates experimental data to determine the extent to which petroleum hydrocarbons pass through the skin. These data strongly suggest that dermal exposure to petroleum hydrocarbons, even following long-term exposures such as in occupational settings, will not cause systemic toxicity under normal working conditions and assuming an intact skin barrier. Skin contact with some petroleum products may cause skin irritation, leading to dermatitis, particularly after repeated or prolonged exposure. In addition to these irritating effects, the skin barrier function may be affected following repeated contact with petroleum hydrocarbons, making the skin potentially more susceptible to other irritants, sensitizing agents, and bacteria. In addition, the impaired skin barrier may lead to increased dermal penetration of hydrocarbons and other substances. To avoid this there is a need to minimise skin contact.

KEYWORDS

Dermal exposure; absorption; permeability; flux; QSAR; skin; risk assessment; petroleum hydrocarbons

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SUMMARY

Commercial petroleum products, such as gasoline, kerosene, and fuel oils, are complex mixtures consisting of hundreds of different hydrocarbons. Dermal exposure to petroleum hydrocarbons, for instance in the work place, may contribute to systemic levels of various aromatic and aliphatic hydrocarbon components. To assess the health risk associated with dermal absorption, in addition to external exposure (i.e. total exposure onto the skin), the extent to which petroleum hydrocarbons pass through the skin needs to be evaluated. Dermal absorption can be assessed either by measurements in *in vivo* in humans, or predicted by using animal, *in vitro*, and *in silico* models (e.g. Quantitative Structure-Activity Relationships or QSARs). One of the specific problems in assessment of the absorption rates of petroleum hydrocarbons is their lipophilicity which is usually expressed based on the partitioning coefficient of the material between *n*-octanol and water (K_{ow}), for instance the log K_{ow}values for toluene and hexadecane are 2.7 and 8.2, respectively (indicating that hexadecane has a much smaller water solubility than toluene. In particular for petroleum hydrocarbons, study design regarding the type of skin preparation, vehicle, and the way dermal absorption is expressed might strongly influence the outcomes. Furthermore, the vast majority of the compounds on which the QSARs are based have log K_{ow} values below 4.4 (i.e. relatively water soluble compounds)). In addition, these models are mainly derived from *in vitro* experiments obtained from aqueous solutions raising the question whether use of these models for the prediction of highly lipophilic petroleum hydrocarbons is justified since humans are rarely, if ever, exposed to aqueous solutions of petroleum hydrocarbons.

The main aim of this report is to critically evaluate the available experimental data on dermal absorption of petroleum hydrocarbons. Furthermore, permeability coefficient (Kp) and maximum flux data predicted by QSARs were compared with experimental values obtained from petroleum hydrocarbons in aqueous solutions, as neat individual hydrocarbons, and as the products themselves. The literature data on Kp and flux obtained from *in vivo* and *in vitro* studies have been evaluated with regard to the vehicle and the type of skin. For prediction of dermal absorption, two QSARs (Episuit-Dermwin and Skinperm) have been used.

The results indicate that the experimentally determined absorption from aqueous solutions were several orders of magnitude higher than the absorption after dermal exposure to either a neat chemical or a petroleum product such as a jet fuel. Furthermore, the experimental K_p values show inverse proportionality with lipophilicity of the hydrocarbons which is in contrast with the trend obtained by both prediction models. The predicted K_p values were similar for both models and showed a significant overprediction when compared with the K_p values obtained in studies performed with petroleum products or neat solvents. It was found that there is a better agreement between predicted and experimental values for aqueous solutions. However, for the maximum flux, a decrease in maximum flux with increasing K_{ow} is obtained for both models, which is similar to the trend obtained in experimental studies.

Overall, it is concluded that the absorption rates of petroleum hydrocarbons from aqueous solutions highly overestimated those from neat petroleum products emphasizing the need for conducting absorption studies under ‘’in use’’ scenarios. Furthermore, the maximum flux that defines the highest dermal exposure risk for a chemical, and QSARs based on this approach, may offer a better approach to dermal risk assessment of highly lipophilic petroleum hydrocarbons.

1. INTRODUCTION

As the first organ in contact with the external environment, the skin can be frequently exposed to various liquid chemicals (by spills, splashes, immersion, or contacts with contaminated surfaces) or vapours. Absorption of a chemical into the skin may lead to local effects such as inflammation or sensitization or to systemic effects after subsequent uptake and distribution by the circulation. In a workplace situation absorption of hazardous substances through the skin can contribute considerably to the total systemic uptake or in some cases even become the main absorption route. To assess the health risk associated with dermal exposure, the extent to which a substance that is deposited onto the skin can be expected to pass through the skin needs to be evaluated.

In the first part of this document an overview has been given of the methodology used for the assessment of dermal exposure and absorption of chemicals, including experimental methods and predictive modelling. Furthermore, test methods for local skin effects such as irritation, allergic sensitisation and corrosion have been addressed.

In addition to methodological issues, this report presents recent literature studies on the dermal absorption and local skin effects of petroleum hydrocarbons.

Although dermal absorption of petroleum hydrocarbons can be determined *in vivo* in human skin, for ethical and technical reasons, various *in vitro* and animal models have been used to predict dermal absorption in humans. Recently, new guidelines for the conduct of these studies have been adopted by regulatory bodies. Among animal models, pig skin is considered the best choice since rat skin over-predicts dermal absorption through human skin. In *in vitro* testing the choice of receptor fluid, type of skin and vehicle have been shown to be particularly important factors for highly lipophilic compounds. For some hydrocarbons including benzene, toluene and xylene the absorption from aqueous solutions was several orders of magnitude higher than that seen after dermal exposure to either a neat chemical or a petroleum product e.g. jet fuel. Since petroleum products are complex mixtures with varying composition, experimental conditions must be relevant for the situation of the worker or consumer.

The most widely applied QSAR models for the prediction of dermal absorption, such as Episuit-Dermwin and Skinperm, relate the permeability coefficient (K_p) to some physicochemical parameters of the compound such as molecular weight (MW), water solubility and lipophilicity (K_{ow}). One of the specific problems in the application of these models for the prediction of dermal absorption of hydrocarbons is that the vast majority of the compounds on which the QSARs are based have P_{ow} (log K_{ow}) values below ~4.4. For the numerous constituents in petroleum products that have P_{ow} values greater than 4.4, the K_p predictions may therefore be less reliable. For highly lipophilic hydrocarbons (P_{ow} > 4), the Skinperm and Dermwin models significantly overpredict the K_p. However, using the Dermwin program to predict the dermally absorbed dose per unit area per event (DAevent) may result in a more realistic estimate of the dermal absorption. Another problem in relation to hydrocarbons is that most predictive models are based on permeability data obtained from aqueous solutions, which were markedly higher than comparable data obtained with neat chemicals or lipophilic mixtures. In the case of petroleum hydrocarbons, the maximum flux provides a better estimate of dermal absorption than K_p. The maximum dermal flux can be estimated by multiplying the permeability coefficient obtained in water by its water solubilty. In the case of mixtures the

maximum flux of each constituent may be considered as a very worst case approach, since we are not dealing with neat solutions but compounds that represent only a fraction of the solution mixture.

In spite of all the shortcomings with the methods for estimating dermal absorption, the presently available models (Quantitative Structure-Permeation Relationships - QSPeRs) can help in defining compounds (solutes) as good, bad or intermediate skin permeants.

Dermal exposure of chemicals can be quantified using various measurement techniques or predicted by using predictive models. The use of these methods in assessment of dermal exposure to petroleum hydrocarbons is hampered by the lack of method standardisation and validation.

An extensive literature review shows that individual petroleum hydrocarbons are able to penetrate into and through the skin when applied as pure substances, in mixtures or as commercial products. Generally, a higher absorption of aromatics (e.g. toluene, xylene, naphthalene) than of aliphatics (decane, dodecane, tridecane and hexadecane) has been reported. Available data on dermal absorption of individual hydrocarbons from petroleum products such as jet fuel, strongly suggest that dermal exposure to these products would not cause systemic toxicity under normal working conditions and assuming an intact skin barrier. Pre-exposure to petroleum products might lead to a higher absorption of the hydrocarbons implying an alteration of the skin barrier by repeated exposure. Individual hydrocarbons as well as petroleum products are also known to cause skin irritation, mainly due to the damaging effect on the skin barrier. In general, aromatic hydrocarbons are more irritating to the skin than aliphatic hydrocarbons. However, aliphatic hydrocarbons are retained longer in the skin due to their lipophilicity and have been shown to cause skin irritation which peaks around tetradecane (C14). Animal studies provide evidence that severe dermal irritation induced by repeated long-term exposure to petroleum fuels can contribute to the progression-promoting effect and development of skin tumours. Experimental data indicate that the tumour-promoting activity of alkanes is related to their chain length, with maximal activity found in C12-C14 alkanes. It is plausible to assume that repeated, long-term exposure to petroleum dermally irritating hydrocarbons may pose a carcinogenic risk, which is minimised if long-term irritation is prevented.

In contrast to the skin irritating effect, there is no evidence that petroleum hydrocarbons cause skin sensitization.

2. METHODS FOR THE ASSESSMENT OF DERMAL ABSORPTION

Dermal absorption can be assessed either by measurements in *in vivo* experimental exposure studies in humans, or can be predicted by using animal, *in vitro* and *in silico* models (e.g. QSPeRs).

This chapter is an overview of the experimental methods available for measuring dermal absorption.

2.1. EXPERIMENTAL METHODS

2.1.1. *In vivo* methods

The most appropriate approach for the studying of percutaneous penetration for risk assessment purposes would be to undertake studies in human volunteers. Since their use is limited and their conduct is closely regulated due to technical and ethical constraints (ICH, 1996; Declaration of Helsinki, 2004), an alternative approach to generate *in vivo* dermal absorption data is to use laboratory animal models. This section gives an overview of *in vivo* methodology for dermal absorption in both human volunteers and in laboratory animals.

2.1.1.1. *In vivo* methods in human volunteers

Human *in vivo* studies are considered as the “golden standard” against which all other alternative methods such as *in vitro* assays and animal models should be judged (Howes *et al*., 1996). The main advantage of human *in vivo* methods is that they allow for the determination of the extent of systemic absorption of the test substance in a natural, unaffected living system.

Generally in *in vivo* studies, the chemical is applied as neat or as a solution in an appropriate vehicle onto a designated surface area of the skin for a defined time period. The chemical is usually applied to the forearm or back skin. For vapours, whole body exposure can also be performed.

Dermal absorption can be assessed using different approaches. Common methods for determination of *in vivo* dermal absorption in humans include the measurement of the parent chemical and/or metabolite levels in biological material (e.g. blood, urine, and exhaled air), the microdialysis technique and tape stripping.

Plasma and/or excreta measurements

The extent of dermal absorption of chemicals can be assessed by measuring the amount of the parent chemical or its metabolite in plasma, exhaled air or urine (Akrill *et al*., 2002; Brooke *et al*., 1998; Cardona *et al*., 1993). The amount of chemical determined after dermal exposure is compared to that after a reference exposure with a known input rate or dose such as intra-venous administration or inhalation (in case of volatile compounds). If the total amount of the chemical (e.g. total urine excretion) or the area under the blood/urine concentration-time profile (Area Under the Curve - AUC) of the two routes is compared, the dermal absorbed dose can be calculated from:

$$\text{Absorbed dose}_{dermal} = (\text{AUC or Excretion}_{dermal}/\text{AUC or Excretion}_{ref})*\text{Dose}_{ref}$$

From the absorbed amount, exposed skin area and exposure duration, the average rate of absorption into the skin throughout the exposure can be deduced:

Absorption $rate_{dermal}$ = Absorbed $dose_{dermal}$/(Area*Time)

Since this absorption rate is not constant during the exposure but dependent on the exposure duration, it cannot easily be extrapolated to other exposure scenarios. For more detailed dermal kinetics, concentration-time profiles have to be determined. Using appropriate kinetic analysis such as the (de)convolution method, dermal absorption rates as a function of time can be deduced from the concentration-time profiles (Opdam, 1991). The advantage of this approach is that, in addition to the average absorption rate into or through the skin, the maximum absorption rate can be deduced and, if a steady state absorption is reached, also the permeability coefficient (K_p) (Opdam, 1991; Kezic *et al*., 2004). This method has been widely used for the determination of dermal absorption for solvents, drugs and other chemicals (e.g. Kezic *et al*., 2001; Jakasa *et al*, 2004).

The measurement of the parent chemical and/or its metabolite in plasma and excreta is of practical importance for risk assessment since it is expected to give an indication of the internal dose which would be expected to give a better correlation for any adverse health effects than external exposure (i.e. total exposure onto the skin). Especially in the case that dermal absorption contributes substantially to the total absorption, estimation of the internal dose by means of biological monitoring has to be preferred to environmental monitoring (since the latter implicitly assumes inhalation as the only route of uptake), particularly when specific markers are available. In addition to the occupational exposure levels for airborne exposure, their biological equivalents known as Biological Exposure Indices have been set for a number of chemicals (ACGIH, 2001).

Microdialysis

Microdialysis is a technique that measures an amount of a dermally applied chemical in the extracellular space beneath the exposed skin site. Recent reviews offer an in-depth view of methodological aspects and applications of the microdialysis technique (de Lange *et al*., 2000; Joukhadar and Muller, 2005; Kreilgaard, 2000; Schnetz and Fartasch, 2001) The principle of the technique is based on passive diffusion of chemicals across the semi-permeable membrane of a microdialysis probe that is introduced into the subcutaneous tissue parallel to the skin surface. The probe is usually perfused slowly with a physiological solution to mimic blood flow. Molecules able to diffuse across the probe membrane can be analysed in the perfusate which is collected at certain time points. The microdialysis technique enables the determination of detailed kinetics without the necessity to perform a reference exposure (Benfeldt *et al*., 2007; Benfeldt, 1999). However, this technique also has limitations. Implantation of the probe can elicit a tissue reaction, which in turn can influence skin absorption (Anderson *et al*., 1996; Schnetz and Fartasch, 2001). Furthermore, only a fraction of the chemical that has penetrated the skin is recovered in the dialysate. The recovery efficiency depends on several experimental factors such as position of the tubing, physico-chemical properties of the chemical and the nature of the perfusate. (Kreilgaard, 2000; Schnetz and Fartasch, 2001). An additional limitation of the microdialysis technique is that it utilizes an aqueous perfusate and can therefore only dialyse water-soluble substances.

Tape stripping

The tape stripping method is based on determination of the amount of chemical in the consecutive layers of the *stratum corneum*[1]. After the end of a dermal exposure period, the *stratum corneum* of the exposed skin site is removed sequentially by adhesive tape. The amount of recovered substance in each tape strip is determined with an appropriate analytical technique. Depending on the exposure period, time of *stratum corneum* harvesting, and the part of *stratum corneum* which is taken for the analysis, different approaches are proposed (Chao *et al*., 2005; Dupuis *et al*., 1984; Mattorano *et al*., 2004).

In some studies, the amount of chemical is measured only in the superficial layers of the *stratum corneum*. It has been reported that the amount of the chemical in these *stratum corneum* layers was a good estimate of the total amount of the chemical absorbed into the systemic circulation (Chao *et al*., 2005; Dupuis *et al*., 1984; Mattorano *et al*., 2004; Rougier *et al*., 1983). The main problem with this methodology is the variability in the amount of the chemical recovered by each tape strip. The recovery is influenced by several factors; type of adhesive tape, vehicle in which the chemical is applied and the applied pressure on the tape prior to removal from the skin site. To avoid this source of variation, the amount of *stratum corneum* could be determined by measuring the weight of the *stratum corne*um in the strips or be estimated indirectly by, for example, the protein content or by assessing trans-epidermal water loss (Dreher *et al*., 1998; Jakasa *et al*., 2007; Pirot *et al*., 1997; Tsai *et al*., 2003; Weigmann *et al*., 2003).

Instead of measuring the total amount of the chemical in the *stratum corneum*, in some studies the concentration profile of the chemical across the entire *stratum corneum* depth has been determined. From the concentration-depth profiles, the diffusion coefficient and partition coefficient of the chemical between vehicle and the *stratum corneum* equation can be deduced which allows estimation of the permeability coefficient. (Alberti *et al*., 2001; Jakasa *et al*., 2007; Pirot *et al*., 1999; Reddy *et al*., 2002)

Although the tape stripping technique has certain advantages, there are critical points. Some authors use the number of consecutive tape strips instead of measuring the real *stratum corneum* depth thereby assuming that the amount of the removed *stratum corneum* by each strip is linearly proportional to the number of strips (Tsai *et al*., 1991). However, the amount of removed *stratum corneum* is known to vary considerably for different individuals and with the depth (Bashir *et al*., 2001; Tsai *et al*., 1991). The homogeneity of subsequent *stratum corneum* layers is also a point of concern: due to the furrows in the *stratum corneum* the amount of the chemical measured in one strip can come from different layers of the skin (van der Molen *et al*., 1997). Furthermore, the time taken to remove the entire *stratum corneum* can be critical for the determination of fast penetrating or volatile chemicals (Reddy *et al*., 2002).

Spectroscopic methods

A variety of spectroscopic methods have been applied in studying *in vivo* penetration of chemicals, with the vast majority being based on infrared (IR) and Raman vibrational spectroscopy (Alvarez-Roman *et al*., 2004; Hanh *et al*., 2000; Touitou *et al*., 1998). The main advantage of these spectroscopic techniques is that

[1] The *stratum corneum* is the outermost layer of the skin and is made of several tight layers of dead, flat skin cells that shed about every 2 weeks; the *stratum corneum* is important for the barrier protection of the skin.

they are rapid, non-invasive, and some of them can provide real time data on chemical penetration through the skin.

The most frequently used IR technique for studying penetration is attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) in which the spectra can be recorded directly from the skin, which is placed onto the ATR crystal. The amount of a test chemical taken up can be quantified as a function of time, by following its disappearance from the most superficial layers of the *stratum corneum* (Mak *et al*., 1990). Since the IR beam has a low penetration depth of only 1-2 µm (Laugel *et al*., 2001; Nothinger and Imhof, 2004), the measurements of IR spectra as a function of different depths are performed in combination with tape stripping. The penetrating substance is measured on the treated site *in vivo* and after each removal of the *stratum corneum* or indirectly on the tape strips (Alberti *et al*., 2001; Pirot *et al*., 1997). ATR-FTIR has been extensively used to quantify the absorption of various drugs, pesticides and other chemicals (Alberti *et al*., 2001; Ayala-Bravo *et al*., 2003; Carden *et al*., 2005; Moser *et al*., 2001; Pirot *et al*., 1997; Reddy *et al*., 2002, Touitou *et al*., 2000).

Rather recently other IR techniques have been introduced such as thermal emission decay FTIR (Notingher and Imhof, 2004), opto-thermal radiometry and mid-infrared spectroscopy (Xiao *et al*., 2001; Ring *et al*., 2006). These techniques show less sensitivity to small movements and roughness of the skin sample. In addition, the spectra can be recorded up to a *stratum corneum* depth of 10 µm without interference from deeper layers. Confocal Raman microspectroscopy represents another recent *in vivo* approach for non-invasive determination of a molecular profile at various depths in the skin (Caspers *et al*., 2001). So far, this technique has been used mainly to identify molecular structures of the skin, and only a few studies have determined the diffusion profile of a chemical in the skin (Caspers *et al*., 1998; Caspers *et al*., 2000; Caspers *et al*., 2002). The fact that the technique is completely non-invasive, that it allows depth-profiling and that its spectrum is highly molecule specific make this technique a promising tool for determination of percutaneous absorption. The main limitation of spectroscopic techniques is that in general the chemical has to have a specific absorption spectrum that is sufficiently distinct from that of the *stratum corneum*.

2.1.1.2. *In vivo* methods in laboratory animals

Although human skin is the best model for human risk assessment and the acceptance of the use of laboratory animals is decreasing, there are several reasons why animal *in vivo* models are still used for dermal risk assessment. Firstly, due to practical and ethical issues, conduct of exposure studies in humans is not always possible. Secondly, *in vitro* tests are not accepted in some countries, and data from animal studies, in particular those obtained from the rat are required for the regulatory risk assessment process. (US-EPA, 1998; Zendzian, 2000) Furthermore, in comparison with *in vitro* tests, an *in vivo* animal model uses a physiologically and metabolically intact system.

In addition to ethical issues, the disadvantage of using laboratory animals is that they have different skin permeability and systemic disposition properties compared to humans. The skin structure differs from one species to another (for example, in the epidermis the *stratum corneum* is thicker in species without hair), between different strains of the same species and even within the same species (for instance, differences in absorption for the various parts of the body) (EU DG SANCO, 2004). While rats, guinea pigs and rabbits have a more permeable skin than humans, the skin absorption properties of monkeys and pigs are reported to be

more similar to that of humans (OECD, 2004c). The results of a species comparative study performed by Bartek *et al*. (1972) indicated that the dermal absorption increases in the following order:

man < pig < rat < rabbit.

It is only recently that the OECD adopted a guideline for *in vivo* dermal absorption (OECD, 2004a,b). This is similar to the guideline for Dermal Absorption Studies of Pesticides in the rat published by the US-EPA (1998); however, the standard OECD protocol requires considerably fewer animals, since the number of exposure intervals and the number of dose levels is lower than in the US-EPA (1998) protocol.

Principles of the standard *in vivo* tests

The experimental protocols for *in vivo* methods have been described in detail in the adopted OECD and US-EPA guidelines, so in this review the principles and methods will only be discussed briefly (EHC, 2006; OECD, 2004a,b; US-EPA, 1986).

There are three classes of *in vivo* animal studies. Studies 1) in which a compound is measured in blood or excreta, 2) in which material is measured in the skin by biopsy or some other method, and 3) in which the compound is measured in all tissues (residue analysis). (EHC, 2006; OECD, 2004a,b; US-EPA,1986)

The test chemical is applied to the designated area of skin in an appropriate form, for a defined period of time. Ideally, a radiolabeled chemical is used.

Selection of animals

The most commonly used animal in dermal absorption studies is the rat because this allows for direct comparison with other *in vivo* studies, which are generally performed in this species (EHC, 2006; Zendzian, 2000). It is generally recognized that rat overpredicts human skin absorption and will therefore lead to a conservative estimate of percutaneous absorption for risk assessment. The factor of difference (the ratio between the values obtained in rat and human skin) appears not to be determined by molecular weight, lipophilicity, or aqueous solubility of a chemical, although in general better agreement was found for less lipophilic compounds. (Van Ravenzwaay and Leibold, 2004 a,b; Vecchia and Bunge, 2005; ECETOC, 1993) Due to inconsistent differences in percutaneous absorption between rat and human skin it is not possible to derive a general adjustment factor for estimation of human percutaneous absorption. However, when percutaneous absorption data are available for rat *in vivo* and for rat and human skin *in vitro*, the *in vivo* dermal absorption through human skin may be estimated from the relationship proposed by Van Ravenzwaay and Leibold (2004 b):

Human *in vivo* = (rat *in vivo*) x (*in vitro* human)/(*in vitro* rat)

Test substance and application

The test substance can be applied neat, diluted or in formulated mixtures to represent as close as possible real human exposure. Vehicles other than water can be used, but their interaction with the substance and absorption characteristics should be known. The amount applied should mimic normal use.

Exposure duration

The OECD guideline (2004 a,b) requires that duration of exposure mimics usual human exposure (typically 6-24 hours) whereas a 24 h termination time point would allow estimation of daily systemic exposure. In contrast, the US-EPA guideline prescribes evaluation of dermal absorption for a series of exposure durations (US-EPA, 1998). After the end of exposure, the skin site is washed in a manner that reflects normal hygiene practice (using aqueous soap) (OECD, 2004 a, b). In the US-EPA (1998) protocol, the animals are sacrificed at this time point. In the OECD (2004b) protocol, the animals are housed individually in metabolism cages after the skin is washed. Excreta and (if appropriate) exhaled air are collected until sacrifice of the animals.

Analysis of data and reporting

The amount of a test chemical determined in blood, excreta, expired air and carcass and the cage after washing enables determination of the amount absorbed at each time point. Usually the dermal absorption is expressed as the percentage of the applied dose. Additional studies can be performed to determine whether test compound remaining in the layers of the skin at the exposure site will be available for systemic absorption or not. In the case of infinite dose, steady state absorption rate and permeability coefficient are determined.

2.1.2. *In vitro* methods

In vitro methods for assessing dermal absorption are based on measuring the diffusion of a test chemical into and across excised skin to a fluid reservoir.

A variety of protocols and experimental conditions have been used for determination of percutaneous absorption *in vitro* and it is only recently that standard guidelines for conducting *in vitro* tests have been adopted (OECD, 2004 a,b). In line with the OECD guidelines the criteria for *in vitro* assessment of dermal uptake of cosmetic ingredients were defined and updated (SCCNFP, 2003; 2006). In addition to these documents, the US Environmental Protection Agency proposed rules for *in vitro* testing of 33 chemicals aimed at evaluating the need for “skin designations” for these chemicals (US-EPA, 2004).

The adopted guidelines allow for some flexibility with respect to, for example, the type and thickness of the skin sample, dose levels, composition of receptor fluid and vehicle. Flexibility in the choice of experimental design was needed to enable measurements to be made under circumstances which are as close as possible to the real life exposure situation. However, experimental conditions such as type and preparation of the skin and the physico-chemical properties of the receptor fluid are known to influence the predictive value of an *in vitro* test for the human (*in vivo*) situation (Jones *et al*., 2004; EHC, 2006; Williams, 2006; Jakasa *et al*., 2007). An additional challenge in the dermal risk assessment process is the interpretation of the results from the *in vitro* study. The data obtained from percutaneous absorption experiments are used in a number of ways for the calculation of dermal absorption parameters and expression of the permeation characteristics. In this respect the question whether or not material retained in the various skin layers should be included in the overall dermal penetration value is still a point of debate. The factors which might influence the predictive value of percutaneous absorption tests have been extensively addressed in recent monographs and reviews (Jones *et al*., 2004; EDETOX, 2004; EHC 2006; Williams, 2006; Jakasa *et al*., 2007).

Principles of the *in vitro* tests

For an *in vitro* skin-penetration study a piece of excised skin is positioned between two chambers of a diffusion cell which may be of either of static or flow-through design (**Figure 1**). The test substance is applied either neat or dissolved in an appropriate vehicle to the skin membrane and remains in contact with the skin for a defined period of time before removal by an appropriate cleansing procedure. The lower chamber (''receptor'') contains a receptor fluid that is designed to mimic the role of the blood *in vivo*. The receptor fluid, containing the test chemical that has passed through the skin, is collected once at the end of the experiment or, preferably, at regular intervals during the exposure period so that a penetration time profile may be constructed. The amount of chemical in the skin and in the receptor fluid can be measured using appropriate analytical techniques such as high performance liquid chromatography, gas chromatography or scintillation counting. The use of radiolabeled chemicals (usually ^{14}Carbon or tritium) is preferred because of the high detection sensitivity, especially if chemical-penetration levels are very low.

Maintaining a constant temperature at 32 ± 1°C (temperature of the skin surface) is important since the rate and the extent of skin absorption is temperature-dependent.

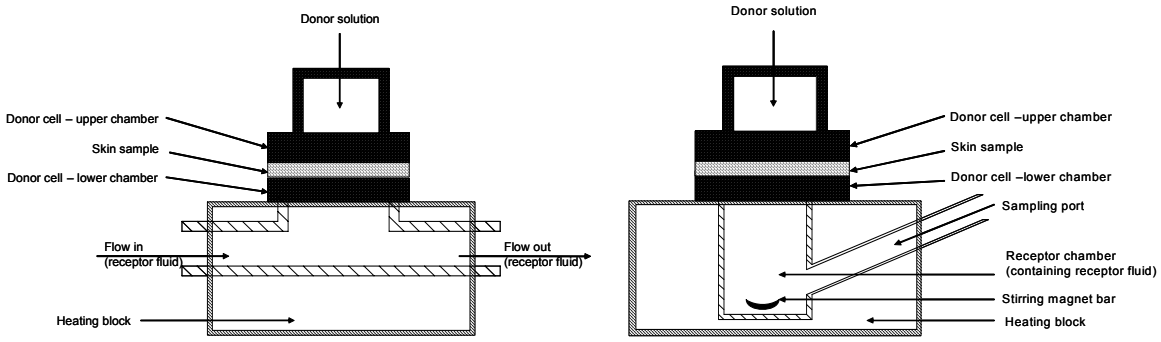
Diffusion cells

Both, static and flow-through diffusion cells are considered suitable (OECD, 2004a,b). The main difference between these two types of diffusion cells is in the design of the receptor compartment. Static cells are usually designed for use with larger skin areas and are equipped with larger receptor chambers with the receptor fluid collected manually through a sampling port (**Figure 1b**). In static diffusion systems it is important to ensure that the test compound is sufficiently soluble in the receptor fluid throughout the exposure period (Brain *et al*., 1998a; OECD, 2004b; EHC, 2006).

There are different types of flow-through diffusion cells, but basically there is a continuous flow of receptor fluid through the receptor cell just beneath the donor chamber (**Figure 1a**). The receptor fluid containing a test chemical is transferred through tubing (usually teflon) into the test vials placed in an automatic collector enabling simultaneous collection from a number of cells.

Figure 1 Schematic flow-through and static diffusion cells for measuring dermal absorption.

(a) Flow-through diffusion cell (b) Static diffusion cell



Flow-through diffusion cells have the advantage that they more closely mimic the *in vivo* blood flow. However, dilution of the sample collected over time, reduces the analytical sensitivity.

Several comparative studies showed no systematic differences in skin absorption obtained with static or flow-through diffusion cells (Bronaugh and Maibach, 1985; Bronaugh and Stewart, 1985; Clowes *et al*., 1994; Hughes *et al*., 1993). From a large multi-center comparison study on absorption of three model permeants: caffeine, testosterone and benzoic acid, using both types of diffusion cells, the design of these cells appeared not to be a significant source of variation (Van de Sandt *et al*., 2004). Using a standard silicone rubber membrane, Chillcot *et al*. (2005) also did not reveal a consistent pattern of differences between the results of static and flow-through diffusion cells.

Some other systems

Although *in vitro* assays are commonly performed using diffusion cells, there are a few other systems that may be very useful for determination of percutaneous absorption. Recently a new *in vitro* technique based on thermal gravimetric analysis has been proposed which appears to be very suitable for measurement of dermal absorption of vapours of volatile compounds such as 2-propanol, methanol and toluene (Rauma *et al*., 2006).

The isolated perfused porcine skin flap (IPPSF) and isolated pig ear are other *in vitro* skin models. The primary advantage of these systems as compared with diffusion chambers is that they allow measurement of percutaneous absorption in a viable skin preparation which has a normal anatomical structure and a functional microcirculation (Riviere *et al*., 1996; 1995; Riviere and Monteiro-Riviere, 1991; de Lange *et al*., 1994). This model has been used for investigation of different classes of chemicals such as xylene, pesticides, etc.

Receptor fluid

One of the prerequisites for an appropriate receptor fluid is sufficient solubility of the test chemical. Furthermore, the receptor fluid should not alter the barrier properties of the skin membrane. For water soluble compounds, the most frequently used receptor fluid is saline solution. To improve solubility of lipophilic compounds, bovine serum albumine (BSA), organic solvents or surfactants can be added to the receptor fluid (Bronaugh *et al*., 1999; Cross *et al*., 2003; de Lange *et al*., 1994). However, organic solvents and surfactants can cause damage of the skin barrier (Sartorelli *et al*., 2000). In general, the choice of the receptor fluid has a more pronounced effect on the dermal absorption of lipophilic compounds due to their limited solubility in most receptor fluids. However, some studies show that the presence of materials such as BSA in receptor fluid can significantly influence dermal absorption of relatively hydrophilic compounds, probably due to protein binding of the test chemical (Wilkinson and Williams, 2002).

Skin type and preparation

OECD and SCCNFP guidelines for *in vitro* testing (OECD, 2004b; SCCNFP, 2003, 2006) allow for the use of human and animal skin, whereas US-EPA (US-EPA, 2004) prescribes the use of human cadaver skin only. In studies using animal skin, pig skin is preferred because it resembles best the permeation properties of the human skin (OECD, 2004; SCCNFP, 2006). Rat skin can also be used, but caution should be taken in the interpretation of the permeation data, since it is known that rat skin is more permeable than human skin (van Ravenzwaay and Leibold, 2004a; Vecchia and Bunge, 2005). Since differences in the percutaneous absorption of

human and rat skin are highly variable and not determined by the physico-chemical properties of a chemical, it is not possible to derive an adjustment factor for human percutaneous absorption from the rat data (van Ravenzwaay and Leibold, 2004a; Vecchia and Bunge, 2002a). Use of artificial skin is not considered appropriate by both SCCNFP and OECD due to insufficient barrier function compared with that of living skin (SCCNFP, 2003, 2006; OECD, 2004b).

For the *in vitro* assay human abdominal or breast skin can be used according to the SCCNFP and OECD guidelines, whereas US-EPA prescribes the use of abdominal skin only. When using pig skin, dermal absorption testing is usually performed with flank or ear skin, although skin from the back or limb is also allowed. In the case of the rat, dorsal and ventral skin may be used.

Skin samples used can be full-thickness (<1000 µm), split-thickness (200-400 µm) and epidermal membrane skin preparations (OECD, 2004b). Full thickness membrane includes, viable epidermis and dermis, whereas in split thickness skin the lower dermis has been removed. Both, the OECD and the SCCNFP guidelines recommend the use of split thickness skin, whereas the use of full thickness skin must be justified. In the US-EPA test rules (US-EPA, 1999, 2004) only split-thickness skin of 200 to 500 µm can be used. In addition to full thickness and split thickness preparations, epidermal membranes comprising the viable epidermis and the *stratum corneum* may be used, but the reason for this choice should be justified (OECD 2004b; SCCNFP, 2003, 2006).

The integrity of the skin sample should be determined prior to the experiment by measuring the penetration of a marker substance (e.g. tritiated water, testosterone) or by measuring TEWL (trans-epidermal water loss) or TER (transcutaneous electrical resistance).

The type of skin preparation has been reported as a major contributor to the variation in results of *in vitro* testing (Ramsey *et al*., 1994; van de Sandt *et al*., 2004; Wilkinson and Williams, 2006). Several comparative studies show that absorption of lipophilic compounds into the receptor fluid is significantly reduced when using full thickness skin as compared to split thickness skin due to the diffusion resistance of the hydrophilic dermis for lipophylic compounds (Wilkinson and Williams, 2002; Yourick *et al*., 2004; Cnubben *et al*., 2002; van de Sandt *et al*., 2004). However, some studies have demonstrated that hydrophilic compounds such as glycol ethers and compounds that can bind to the skin can be retained in the skin revealing a more complex relationship between skin thickness, lipophilicity and dermal absorption (Wilkinson and Williams, 2005).

Vehicle

A test chemical can be applied onto the skin as neat, diluted (ideally with water), or as a formulation (OECD, 2004b; US-EPA, 2004). For lipophilic compounds, use of isopropyl myristate has been proposed (US-EPA, 2004). The physico chemical properties of a vehicle and the solute determine the partitioning of a test chemical in the *stratum corneum* and thereby the dermal absorption *in vivo* as well as *in vitro*. Furthermore, a vehicle can alter the composition and structure of the skin which might lead to enhancement or suppression of dermal absorption (Davis *et al*, 2002). The effect of the vehicle on percutaneous absorption has been demonstrated for compounds of different classes of compounds such as dodecane and naphthalene (Baynes *et al*., 2001), PAH (Sartorelli *et al*., 1999, 2001a), terpenes (Cal, 2006), benzene (Blank and MacAuliffe, 1985), toluene (Boman and Maibach, 2000), lindane (Dick *et al*., 2004), benzoic acid, caffeine and testosterone (Bronough an Franz 1986), glycol ethers (Traynor *et al*., 2007), and agrochemicals (van der

Merwe and Riviere, 2005). Due to the pronounced effect of a vehicle on percutaneous absorption, a test chemical should be applied in the same vehicle/formulation as for “in use” conditions (OECD, 2004 a,b; SCCNFP 2003).

Dose

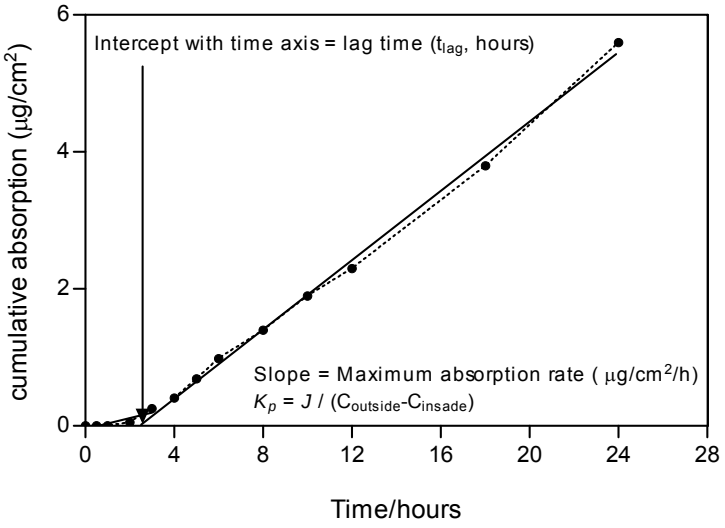
The dosing regime in *in vitro* assays should be as close as possible to the real life exposure. The *in vitro* testing can be performed under finite and infinite dose conditions. In the infinite dose regime, the concentration of a test chemical does not significantly diminish during the dermal exposure duration. The finite dosing regime often better reflects the actual “in-use” scenarios, e.g. short skin contact due to splashes or exposure to volatile compounds which evaporate easily from the skin (OECD, 2004). When exposure duration is sufficiently long to attain steady state absorption, experiments using infinite dose enable the determination of the permeability coefficient, K_p.

Data analysis

After finite dosing, dermal absorption can be expressed as an absolute amount absorbed (µg/cm^2 of skin surface), as the percentage of the applied dose (relative absorption), or as maximum absorption rate (flux, µg/cm^2/h) attained. To calculate the relative absorption, the amount of a test chemical which is washed from the skin should be measured as well as the amount present in the different skin layers and in the receptor fluid. There is still some scientific debate whether the fraction of compound remaining in the skin after washing (rinsing) and at the end of the experiment should be considered as systemically absorbed or not (OECD, 2004b; EHC, 2006; SCCNFP, 2003,2006; CEFIC, 2004). This is particularly relevant for very lipophilic compounds which have a tendency to remain in the *stratum corneum*, but also for other chemicals which may be retained in the skin due to, for example, binding to skin proteins. According to the OECD Test Guidelines, the test substance remaining in the skin should be considered as absorbed unless it is demonstrated that absorption can be determined from receptor fluid only (OECD, 2004b). However, when the test substance remains in the skin at the end of the study, it may need to be included in the total amount absorbed. The guidelines adopted by the European Cosmetic, Toiletry and Perfumery Association (COLIPA) and of the European Commission's Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP, 2003; 2006) state that the amount of a chemical present in the *stratum corneum* at the end of the exposure should not be considered as systemically available. Both guidelines consider the amount of a chemical in the epidermis and dermis and in the receptor fluid as systemically available.

For infinite dose applications, the maximum absorption rate (maximum flux) can be determined and in this case the relative amount absorbed is not relevant. The maximum absorption rate can be deduced from the slope of the cumulative absorption-time profile in its linear part (**Figure 2**). In the case of steady state absorption, the permeability coefficient (K_p, cm/h) can be calculated from the maximal absorption rate. There is usually an interval between the time of applying the dose and the steady state being reached. This “lag time” can also be derived from a graph of the cumulative absorption versus time, and is the intercept (on the time axis) of the tangent to the linear part of the absorption profile.

Figure 2 Cumulative absorption of a test chemical in time. Absorption (y axis) is expressed as the amount penetrated the receptor fluid in mass per unit exposure area)



The use of *in vitro* dermal absorption data in the risk assessment

In vitro studies to determine dermal absorption of chemicals are increasingly used for regulatory purposes. Furthermore, *in vitro* data are used to develop QSARs. Several important factors should be taken into consideration when planning dermal absorption studies *in vitro*. Among these are the choice of vehicle and receptor medium, the choice of skin membrane, and the dosing regime. The experimental conditions regarding dose, vehicle and exposure duration should be aligned with real-life exposure scenarios. On the other hand, the choice of skin membrane and receptor fluid will mainly be determined by the physico-chemical properties of a test chemical. This will be more critical for very lipophilic substances because of their low solubility in most receptor fluids. Within the EU, there is a Technical Guidance Document (EC, 2002) which provides generic guidance on risk characterisation undertaken within the New Substances, Existing Substances Regulation (ESR) and Biocides Product Directive (BPD) legislation. In this document, the problem of choosing a skin reservoir for very lipophilic compounds is indicated, such that there is a justification for the inclusion of material residing within the skin layers in the overall estimate of skin absorption for such substances. By including the amount retained in the skin as absorbed, results from *in vitro* studies seem to correlate with those from *in vivo* experiments and support their use as a replacement for *in vivo* testing (EC, 2003; EHC, 2006; Yourick *et al*., 2004).

For risk assessment purposes, dermal absorption studies with human skin are preferable. When only rat *in vitro* dermal absorption studies are available, the most conservative approach would be to assume that human skin absorption would be equal to rat dermal absorption.

2.1.3. Methods used for petroleum hydrocarbons

The dermal absorption of petroleum hydrocarbons has been investigated using a variety of methods, including *in vivo* studies in human volunteers, animal and *in vitro*

models. For the detection of dermal absorption of hydrocarbons in petroleum products there are several approaches. The first approach involves addition of one or more radioactive constituent hydrocarbons as markers in the product and monitoring the penetration of radioactivity through the skin. For hydrocarbons which penetrate the skin in sufficient amounts to be detected by conventional techniques, radiolabeling is not necessary. Another approach to determine dermal absorption of hydrocarbons is to apply the individual chemical neat or as a (aqueous) solution. Use of various experimental designs concerning type of skin (full thickness vs. epidermal membrane), vehicle, receptor fluid, animal species, dosing conditions (finite vs. infinite dose) makes comparison of the results of the studies extremely difficult. Petroleum hydrocarbons are lipophilic chemicals and as discussed above, it can be reasonably expected that experimental conditions will have a major influence on their absorption. The differences in experimental design are probably one of the reasons for the enormous discrepancies in dermal absorption of various petroleum hydrocarbons reported in literature (see Chapter 4). In general, based on comparative studies for various classes of chemicals it may be assumed that rat studies will give a conservative estimate of human dermal absorption and that the difference will probably be higher for more lipophilic hydrocarbons. For toluene and xylene, rat skin was shown to be up to 10 times more permeable than human skin. For other petroleum hydrocarbons no comparative studies have been reported.

As discussed above, the choice of a vehicle might have considerable influence on the dermal absorption of a chemical. Dermal application of a lipophilic hydrocarbon in water or in other hydrophilic solvent will favour its partitioning into the lipophilic *stratum corneum* hence increasing its dermal absorption. Dermal absorption of benzene, toluene, and xylene from aqueous solution was up to 3 orders of magnitude higher in comparison to the dermal absorption from gasoline or Jet fuel. So, it should be realised that dermal absorption from one vehicle cannot be assumed to be predictive for that from a different matrix. Furthermore it should be kept in mind that most QSAR models used for the prediction of dermal absorption are based on experimental data obtained with aqueous vehicles. In addition to thermodynamic considerations of a vehicle resulting in altered partitioning of a penetrant into the skin, some vehicles can damage the skin barrier leading to higher absorption. Repeated exposure to jet fuels has been demonstrated to cause increased absorption of some aromatic and aliphatic hydrocarbons.

One of the major factors affecting *in vitro* dermal absorption results is the choice of receptor fluid. Hence, for highly lipophilic petroleum hydrocarbons sufficient solubility of a tested hydrocarbon in receptor fluid should be provided. As discussed above, skin reservoir effects can play a significant role for lipophilic compounds. For this reason it is important that the amount of a penetrated hydrocarbon is determined in different skin layers and included in the calculation of dermal absorption.

In conclusion, experimental conditions, among which the choice of vehicle seems to be of particular importance, can influence the results of dermal absorption studies by several orders of magnitude. This emphasizes the need for using exposure and dosing regimes which are closely related to the workplace situation and to further study the dermal absorption of complex mixtures.

2.1.4. Summary of the experimental methods for assessment of dermal absorption

In the assessment of the dermal absorption properties of specific compounds, in either neat or diluted form or in mixtures or formulations, both *in vivo* and *in vitro* studies can be applied. The most reliable dermal absorption data for health risk assessment are obtained from studies in human volunteers, although for technical and ethical reasons their conduct is limited and closely regulated. *In vivo* methods which are based on measurement of the chemical (or radioactivity) in plasma and/or excreta have an additional practical importance for risk assessment of dermal exposure. The levels of chemicals and metabolites give an indication of the internal dose and are as such can be a better correlate for adverse health effects than the external exposure. Recent developments in applying techniques such as *stratum corneum* tape stripping, FTIR and Raman confocal spectroscopy offer the possibility to study *in vivo* dermal absorption in humans in a minimally invasive manner. However, these techniques are not suitable for all classes of chemicals. The tape stripping technique is not well-suited for volatile and rapidly penetrating compounds and still needs thorough standardization and validation. The main limitation of spectroscopic techniques is that the chemical has to have a specific spectrum.

One of the most appropriate animal models for the assessment of human skin absorption is the domestic pig both *in vivo* and *in vitro*. Traditionally, for the purpose of risk assessment, dermal absorption is most often determined using the *in vivo* rat model. However, the majority of data in the published literature show that rat skin is more permeable to chemicals than human skin. Thus the systemic exposure of humans may well be overestimated if the estimation of dermal absorption is based on the results of an *in vitro* or an *in vivo* rat study. Typically, rat skin shows 2-fold higher permeability than human skin, however, for some chemicals this difference has been shown to be significantly higher, being the highest for lipophilic compounds (in some studies up to 40-fold). Due to inconsistent differences in absorption between rat and human skin it is not practical to simply derive a general adjustment factor for estimation of human uptake. However, when absorption data are available for rat *in vivo* and for rat and human skin *in vitro*, the *in vivo* human uptake may be accurately estimated. *In vitro* studies to determine dermal absorption of chemicals are increasingly used for risk assessment of pesticides, biocides, cosmetic ingredients and industrial chemicals in the EU. Although recently *in vitro* skin absorption test guidelines have been adopted, there are still a number of issues that are not precisely defined, raising the questions of the way in which *in vitro* data should be used for dermal risk assessment. Since experimental conditions regarding dose, vehicle and exposure duration are known to influence dermal absorption, the experimental design should be governed by real-life exposure scenarios. In general, the agreement between *in vitro* and *in vivo* is better for hydrophilic than for lipophilic compounds. Most studies show that the use of full thickness skin results in a lower absorption of lipophilic compounds into the receptor fluid when compared with the results obtained with split thickness skin, indicating a reservoir effect in the skin for these compounds. The reservoir effect is also affected by the choice of the receptor fluid. Again, this will have larger impact for lipophilic substances because of their low solubility in most commonly used receptor fluids. To compensate for the amount of chemical retained in the skin at the end of the exposure period, recent guidelines propose inclusion of the amount retained in the epidermis and dermis as being absorbed as a conservative assumption. On the other hand, the amount of chemical present in the *stratum corneum* at the end of the experiment is considered as non-absorbed in most guidelines.

2.2. MODELS FOR THE PREDICTION OF DERMAL ABSORPTION

Introduction

The rather cumbersome procedures needed in the assessment of the dermal absorption properties of hazardous chemicals and the vast amount of chemicals that remain to be tested has resulted in several studies to find ways in which the dermal absorption can be predicted from the intrinsic properties of the compounds of interest and the conditions under which the dermal exposure takes place.

In this chapter a brief introduction and overview will be presented of the various mathematical approaches that have been developed and are used to assess and predict the degree of dermal absorption. The models are based on the experimental data available for a large number of diverse compounds and knowledge of the structure and properties of the skin barrier. Recently comprehensive reviews and reports have been published in which the various aspects of these approaches are discussed in more detail (WHO, 2006; Jones *et al*., 2004; Yamashita and Hashida, 2003). Also in the text the reader is referred to comprehensive reviews on the various subjects.

First of all, the main aspects playing a role in dermal absorption will be discussed briefly. A general overview of the various aspects playing a role in percutaneous absorption has been presented in ECETOC Monograph No. 20 (ECETOC, 1993). The theoretical aspects of skin permeability in relation to percutaneous absorption studies have been outlined in detail by Dugard (1987).

In the dermal absorption and uptake process, the *stratum corneum* layer is generally assumed to form the main barrier, but other layers like the viable epidermis may in some cases (especially for highly lipophilic compounds) also play an important role (see e.g. Cleek and Bunge, 1993; Bunge *et al*, 1995). The partitioning between the external exposure medium and the skin, and the diffusion process through the skin barrier are the main two physicochemical processes underlying dermal absorption.

The mathematical models developed to describe dermal absorption are explicitly or implicitly based on these physicochemical processes and furthermore on a more detailed description and understanding of the structure and properties of the skin. (See e.g Cleek and Bunge, 1993; Bunge *et al*., 1995; Frash and Barbero, 2003; Kasting 2001; Kasting and Miller, 2006; Anissimov and Roberts, 1999, 2001; Kruse *et al.*, 2007).

The transport properties through the skin are usually described by the permeability coefficient K_p which, according to Fick's first law, is defined as:

$$K_p = J_{ss} / (C_d - C_r)$$

where J_{ss} is the steady state flux (i.e. the maximum flux after reaching equilibrium) through the skin and C_d and C_r are the concentrations of the permeant in the donor (exposure vehicle) and receptor (systemic circulation or receptor cell in case of *in vitro* studies), respectively. For a diffusion-based model in which the *stratum corneum* is assumed to represent the barrier for absorption, K_p may be rewritten as:

$$K_p = K_{sc/d} \cdot D_{sc} / h_{sc}$$

where h_{sc} is the apparent thickness of the *stratum corneum*, D_{sc} is the effective permeant diffusivity in the membrane and $K_{sc/d}$ is the partition coefficient between

the *stratum corneum* and the vehicle. Usually, the concentration C_r (i.e. in the systemic circulation or receptor cell) is assumed to be zero or at least negligible compared to C_d (often referred to as the sink condition). Then the steady state flux J_{ss} becomes:

$J_{ss} = K_p \, . \, C_d$

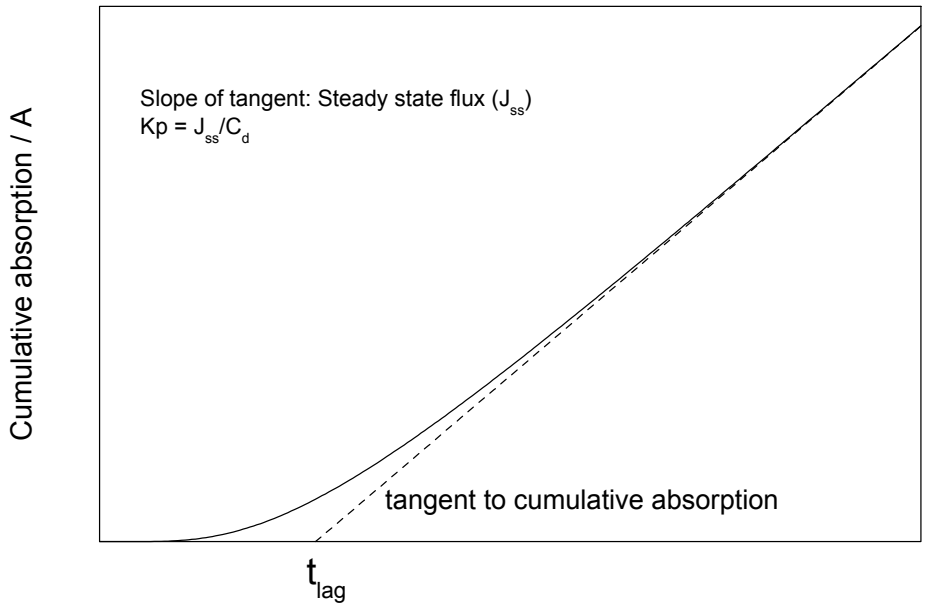
If the concentration in the vehicle (C_d) does not decrease during exposure (so-called infinite dose) the permeability coefficient can be calculated from the donor concentration and the steady state flux. If, on the other hand, the concentration in the donor fluid decreases due to uptake in the skin (finite dose condition), the maximum (steady state) flux may only be reached for a limited time, and may not be maintained or even reached at all. This hampers a proper determination of the permeability coefficient (K_p).

In the first period of dermal exposure there is no equilibrium flux across the skin barrier (no steady state absorption). This non-steady state period is characterized by the lag time (t_{lag}), which for a single homogeneous barrier (e.g. the *stratum corneum*) is given by:

$t_{lag} = (h_{sc})^2/(6. D_{sc})$

The lag time can be also derived from the data generated in a dermal absorption study by determining the time intercept of the tangent to (or practically the linear portion of) the cumulative absorption time course (see **Figure 3**).

Figure 3 Cumulative absorption through a skin area (A). From the tangent to the absorption curve the (maximum) steady state flux (J_{ss}), the permeability coefficient and the lag time are derived as indicated in the figure.



Use of a finite dose (see above), and also the fact that due to longer lag times the steady state has not (yet) been reached may in practice hamper a straight-forward interpretation of the experimental observation in terms of K_p.

In the dermal permeation models $K_{sc/d}$ is usually related to the octanol-water partition coefficient, while D_{sc} is assumed to depend predominantly on the size of the molecule.

For the modelling of dermal absorption, two main approaches can be distinguished: QSARs and mathematical models in which the partitioning and transport processes involved in the dermal absorption are described (Fitzpatrick *et al*., 2004).

In the QSAR, or more specifically QSPeR, approach relationships are established between the permeability properties (usually the permeability coefficient, K_p) and the physicochemical properties of the permeant. To derive these relationships databases containing experimentally determined K_p values for molecules with a range of physicochemical properties (molecular size and lipophilicity) are used. (Vecchia and Bunge, 2002a; see also section 2.2.5 below). The relationships can therefore be used to predict the permeability of untested compounds.

In a second approach, various mathematical models incorporating a more detailed description of the skin structure, the related barrier properties and the resulting transport processes are developed. This approach may result in further insight on the mechanism of skin permeation. The models can be used in the analysis, interpretation and extrapolation of results from dermal absorption experiments, such as time courses of permeation and amount of absorption.

In the following sections the two approaches indicated above (QSPeR and mechanistically based mathematical models) will be reviewed and discussed further. In addition, in the literature some special models for the prediction of dermal absorption are presented, which although related to the previously mentioned approaches, will be described in a separate section (see Section 1.2.4).

2.2.1. QSPeR methods

QSPeRs for dermal absorption are normally based on statistically determined correlations between the physicochemical properties of a range of permeants and solvents and their experimentally determined (steady state) permeability coefficients (Geinoz *et al*., 2004, Potts and Guy, 1992).

QSPeRs have been the subject of several comprehensive reviews (Geinoz *et al*, 2004; Moss *et al.*, 2002; Walker *et al*., 2003; Vecchia and Bunge, 2002a,b). Many of the published QSPeRs are based on a set of permeability coefficients for 94 compounds, published by Flynn (1990).

Based on the Flynn dataset, Potts and Guy (1992) established a QSPeR using a combination of the octanol-water partition coefficient (K_{ow}) and molecular weight (MW) -and thus indirectly the molecular volume - as physicochemical descriptors. It has the following form:

$\text{Log}\ (K_p) = 0.71\ \log\ (K_{ow}) - 0.061\ MW - 2.44$ (with K_p in units of cm/h)

Following this study several molecular descriptors in combination with various permeability databases have been used in studies aimed at optimization of the

QSPeRs. A selection of these studies is given by Fitzpatrick *et al*. (2004). An example of this more complex approach is presented by Patel *et al*. (2002). They recently developed a QSPeR that contained, in addition to terms for hydrophobicity (K_{ow}) and molecular size (MW), two additional calculated descriptors, which were identified to be the most significant in a group of 169 physicochemical descriptors, calculated for compounds in the dataset. The resulting QSPeR has the following form:

$$\text{Log } (K_p) = 0.652 \log (K_{ow}) - 0.0603\ MW - 6.23\ ABSQon - 0.313\ SsssCH - 2.30$$

with

ABSQon the sum of absolute charges on oxygen and nitrogen, and

SsssCH the sum of E-state indices

The various attempts to arrive at an optimal QSPeR may be severely hampered by the quality of the databases. In a recently published report, based on a Workshop organized by CEFIC, the importance of the quality of databases comprising measured and well defined dermal absorption data is emphasized, as a key first step in QSPeR development (Jones *et al*., 2004). The OECD test guidelines for *in vitro* and *in vivo* dermal absorption experiments (OECD, 2004 a,b,c) leave room for variation in the experimental conditions. For further development and improvement of QSPeRs an optimal data base containing uniformly obtained (*in vitro*) results is essential. Therefore it is important to establish and follow strict experimental protocols, as recently proposed in the CEFIC Workshop meeting (Jones *et al*., 2004).

Another problem with most available QSPeRs is that the resulting predicted permeability coefficients (K_p) are not directly applicable in risk assessment, since the permeability coefficients are derived using studies that are (in theory) based on maximum flux (steady state) data obtained from infinite dose *in vitro* studies. However, realistic risk assessment scenarios usually correspond to finite dose conditions and often exposures for limited periods (see e.g. Jones *et al*., 2004).

A further limitation to the application of available QSPeRs lies in the fact that they are based on data obtained using aqueous solutions of single compounds. This hampers their application for compounds present in mixtures and other solvents and for neat (liquid) chemicals.

Recently some critical evaluations of QSPeRs were performed. Geinoz *et al*. (2004) suggested that these models are more reliable when their application is confined to well-defined chemical classes, and that their applicability is restricted by the limited range of polarity and size of the permeants on which they are based. Bouwman (2006) presented an evaluation of 14 QSARs for skin penetration. Many models showed poor external predictivity. Only the models of Patel *et al*. (2002) for K_p and Magnusson *et al*. (2004a) for Jmax provided reasonable predictions for liquids tested neat. They concluded that the values obtained may not be truly applicable for risk assessment.

2.2.2. Mechanistic models

In mechanistically based mathematical models for dermal absorption the transport of a chemical through the skin is related to its structure, the physicochemical

properties of the permeant in relation to the various compartments (layers) in the skin, the vehicle used and, in the case of *in vitro* experiments, also the experimental conditions, such as vehicle volume, receptor volume/flow etc.

In the models that have been developed the skin is assumed to consist of one or more layers, usually the *stratum corneum* and the viable epidermis. The two main processes in the dermal absorption process are:

- the partitioning of the permeant between the vehicle / donor compartment, the various skin layers, and receptor compartment / systemic circulation
- the diffusion through the various skin layers

The processes are described by mathematical equations that are solved in various ways. Reviews of mechanistically based mathematical models in percutaneous absorption have been presented by Roberts *et al*. (1999), McCarley and Bunge (2001) and Roberts and Anissimov (2005).

Some characteristic examples of (recently) developed mathematical models for dermal absorption are described briefly as follows:

Bunge and coworkers (Cleek and Bunge, 1993; Bunge and Cleek, 1995; Bunge *et al*., 1995) developed a model which included consideration of the barrier effect of the viable epidermis for lipophilic compound. They also presented a strategy for estimating the compound specific parameters when using aqueous vehicles. The model was included in documents by the US-EPA (1992). US-EPA (2004) introduced a modification to this model that takes into account losses of lipophilic compounds, which are absorbed in the s*tratum corneum* and lost via desquamation. Furthermore, it was used in estimating the dermal absorption by the DERMWIN module of the Episuite program (Dermwin, 2007).

Anissimov and Roberts developed a pair of models for analysing the effects of flow rate, receptor sampling rate, and viable epidermis resistance for a constant donor concentration (Anissimov and Roberts, 1999, 2000) and the effects of finite vehicle volume and solvent deposited (Anissimov and Roberts, 2001).

Recently a detailed dermal permeation model was published in which, in addition to finite/infinite dosing and non-steady state situations, the effect of repeated exposures and other occupationally relevant exposure scenarios could be simulated (Kruse *et al*., 2007). This model and a new implementation of the finite dose model, based on the publications of Anissimov and Roberts (1999, 2000, 2001), showed good agreement. The models were used for a detailed analysis and interpretation of *in vitro* dermal permeation data (Kruse *et al*., 2007).

Kasting developed models for the analysis of the kinetics of finite dose dermal absorption of the non-volatile compound vanillylnonanamide (Kasting, 2001) and for volatile compounds (Kasting and Miller, 2006).

Frasch and Barbero modelled the steady state flux by describing the *stratum corneum* barrier as a brick and mortar structure and as a more complex irregular structure. The results compared well with those obtained with a homogeneous *stratum corneum* layer (Frasch and Barbero, 2003).

For the analysis of dermal absorption data in human volunteers that were exposed to jet fuel and prediction of blood concentrations following exposure, Kim and

coworkers (Kim *et al*., 2006a) developed a dermatotoxicokinetic model, which included 5 compartments (surface, *stratum corneum*, viable epidermis, blood and storage). The rate constants in the model were determined by fitting to the data. In this approach no physicochemical parameters (diffusion and partition coefficients) were determined.

Other examples of mechanistic modelling are: Roy *et al*. (1996), van der Merwe *et al*. (2006) and Auton *et al*. (1994).

The mechanistic modelling approach may result in further insight in the mechanism of skin permeation. The models can be used in the analysis, interpretation and extrapolation of results of dermal absorption experiments, such as the time course of permeation and amount of absorption. Furthermore, using these models, the effects of finite and infinite dosing can be evaluated. They may also be helpful in the extrapolation of experimentally obtained results to other (occupationally relevant) exposure conditions and scenarios and in the optimization of experimental conditions.

2.2.3. Default values

A third approach is the use of default-values for percutaneous absorption, which are based on theoretical considerations that there should be an optimum in log K_{ow} and a maximum in molecular weight for facilitating percutaneous absorption. De Heer *et al.* (1999) proposed the following criteria to discriminate between chemicals with high and low dermal absorption:

1. 10% dermal absorption for chemicals with a molecular weight > 500 Da and log K_{ow} smaller than -1 or higher than 4, otherwise

2. 100% dermal absorption is used.

In the absence of (reliable) experimental dermal absorption data, this approach is used by the European regulatory authorities involved in risk assessment (EC, 2003, 2004). If the risk assessment outcome based on these default values indicates that the exposure level is acceptable/tolerable, no additional quantitative assessment of the dermal absorption is needed. The advantage of the use of default values is the practical applicability of this approach. On the other hand it is generally considered to result in very conservative (worst case) estimates of dermal absorption.

2.2.4. Specific approaches

In the predictive modelling of dermal absorption some alternative approaches can be distinguished that cannot directly or uniquely be categorized as QSPeR methods or mechanistic models and therefore are discussed in a seperate section.

Maximum flux

The worst case dermal exposure risk for a chemical is defined by its maximum flux (J_{max}) through the skin. Since the maximum dermal flux should occur at the solubility limit of the chemical, it can be estimated as the product of the solubility limit of the chemical in water and its permeability coefficient from water (Roberts *et al*., 2002). Maximum flux values can also be estimated by using QSAR relations. Magnusson *et al*. (2004a) showed that from analysis of a database containing J_{max} values of 278 compounds, MW turned out to be the main determinant for the dermal flux.

Log J_{max} = -4.52 -0.0141 MW (with J_{max} in units of mol /(cm^2.h)

Maximum dermal flux QSARs apply to all vehicles that do not affect the skin integrity. Predictions of rates below the maximum flux can be made on the basis of concentrations expressed in terms of fractional solubilities of a given vehicle (Roberts *et al*., 2002).

Neural networks

Degim *et al*. (2003) described the use of artificial neural network (ANN) modelling for the prediction of dermal absorption. Their work is based on the use of partial charge, log K_{ow} and MW data as input factors into an ANN for the prediction of permeability coefficients. The ANN model successfully predicted experimentally determined permeability coefficients. No direct relationship was observed between descriptors and permeability, indicating the existence of a complex relationship between the structure of the permeant and its dermal permeation.

Skinperm

In the Skinperm model developed by Wilschut and ten Berge (Wilschut *et al*., 1995) the skin barrier is considered to be formed by a protein layer in the s*tratum corneum*, a lipid layer of the *stratum corneum* and an aqueous layer below the *stratum corneum*, through which the substance has to permeate into the capillary bed before entering the bloodstream. The two layers in the *stratum corneum* are assumed to form parallel routes in the permeation process. The overall permeation coefficient from aqueous solution is expressed mathematically as a complex of permeation coefficients representing the permeation through the sub-parts of the skin barrier. The values of the five regression coefficients in the model were derived from a dataset of 123 measured permeability coefficients for 99 different chemicals, and includes the Flynn data set (Wilschut *et al*., 1995). The model can be used to estimate the dermal uptake from both aqueaous solutions and air for various exposure durations. An actualized version of the model (Skinperm 3.3) is available (ten Berge, 2007).

2.2.5. Databases

In the development of QSPeRs the availability of good quality skin permeability data sets is essential. In this section a brief overview of some relevant data sets will be presented. One of the first skin permeability databases used in the development of several QSPeRs was published by Flynn (1990) and contained 97 permeability coefficients for 94 compounds.

For homologous or closely related series of molecules, several permeability data sets have been published:

n-Alkanols:	Scheuplein and Blank (1971, 1973); Flynn and Yalkowsky (1972); Wiechers (1989)
Ethyl ether, 2-butanone, 1-butanol, 2-ethoxyethanol, 2,3-butanediol:	Blank *et al.* (1967)
Phenolic compounds:	Roberts *et al.* (1977)
Glycol ethers:	Dugard *et al.* (1984)
Aromatic amines:	Levillain *et al.* (1998)
Para-substituted phenols:	Hinz *et al.* (1991)
PAHs:	Roy *et al*. (1998); van Rooij *et al*. (1995)

List in adapted form taken from WHO (2006)

In the context of the EDETOX project (EDETOX, 2004) a well documented database was set up, including *in vivo* and *in vitro* percutaneous absorption and distribution data (Soyei and Williams, 2004). The database is accessible via http://www.ncl.ac.uk/edetox.

Recently Vecchia and Bunge (2002a,c) presented detailed summary reviews of published data sets of skin permeation coefficients and s*tratum corneum*/water partition coefficients.

In some recent publications the data sets that were used in the development of QSPeRs have been critically reinvestigated and reanalysed (see e.g. Moss *et al*., 2002; Patel *et al*., 2002; Fitzpatrick *et al*., 2004; and WHO, 2006 - chapter 10).

A maximum flux database for 278 compounds has recently been described and is available as supplemental material from the Journal of Investigative Dermatology (online) at: http://www.nature.com/jid/journal/v122/n4/suppinfo/5602287s1.html?url=/jid/journal/v122/n4/full/5602287a.html. The criteria for inclusion of these compounds are included in the accompanying paper (Magnusson *et al*., 2004a).

2.2.6. Summary of the models for the prediction of dermal absorption

The results of a large number of human *in vitro* studies have been used to derive the chemical permeability coefficients that form the basis for the development of various QSPeRs. The most widely applied QSPeRs relate the K_p to simple physicochemical characteristics for the compounds, such as the MW and the lipophilicity ($\log K_{ow}$). The QSPeRs are usually based on data obtained with aqueous vehicle solutions, and therefore these QSPeRs should in first instance only be used for an indication of the permeability properties from aqueous solutions. Furthermore, the permeability coefficients only relate the concentration in the vehicle on the skin to the dermal permeation rate for steady state and infinite dose conditions.

In practice the QSPeR approach provides predictions of limited accuracy. In part this may be due to the quality of the underlying databases. A more standardized approach to the performance of human *in vitro* studies, like that proposed by the OECD (OECD, 2004c), may help to reduce the variation in the key data used for the QSARs. However, in interlaboratory studies, it has proved difficult to remove all variations.

The permeation data on which the QSPeRs are based are mostly derived by using human skin samples obtained from abdomal skin and breast corrections. Because of the inherent variability in the barrier properties between different skin areas, one must be aware that the QSPeRs based predictions may either over- or underestimate the skin permeability for specific skin areas.

One of the specific problems in applying the available QSPeRs and programs for the prediction of K_p and dermal absorption, such as Episuit-Dermwin and Skinperm that are implicitly using QSARs, is that the vast majority of the compounds on which the QSARs are based have P_{ow} values below about 4.4. For the numerous constituents in the petroleum products that have P_{ow} values greater than 4.4, the K_p predictions may therefore be less reliable. In this respect it must also be noted that accurate determination of the K_p is in practice very difficult to achieve for these type of compounds (high log K_{ow}) because infinite dose is difficult to achieve when using limited aqueous vehicle volumes (Kruse *et al*., 2007).

In general the QSPeRs and modelling programs based on these QSPeRs (Skinperm, Dermwin) predict an increase in K_p with increasing K_{ow} (at fixed MW).

This may result in predicted K_p ranging from 1-10 cm/h for log K_{ow} > 6. This is in sharp contrast with practical and experimental observation, which show that highly lipophilic compounds hardly permeate the skin (but stay in the lipophilic skin reservoir), and theoretical considerations on the hydrophilic barrier which the viable epidermis presents to lipophilic compounds (Cleek and Bunge, 1993). In the Dermwin program the dermally absorbed dose per unit area per event (DAevent) based on the approach of Cleek and Bunge (1993) may be used to obtain a more realistic idea of dermal absorption and the absorption rate per exposure event.

Even if the QSPeRs might be improved by using more recent and better data, they may only apply to one or a few situations (e.g. infinite dose and steady state) and complex extrapolation procedures should be performed to deal with actual exposure conditions.

In particular, application of the K_p may be a problem in the case of relatively long dermal permeation lag times (e.g. in case of high MW molecules) and/or short (acute) exposure durations. In these cases the total penetration (amount absorbed into the skin) will be much larger than that based on the K_p predictions, while the permeation rate (absorption rate through the skin into the system) will be much lower than predicted from the steady state permeability coefficient.

The expression of toxicity is a function of the dose at the target site. This dose may be the concentration (C_{max} at the target site) or the total amount delivered (for example. AUC at the target site) of the parent compound or a relevant metabolite. With respect to assessing the systemic risk of dermal permeation, two parameters may be distinguished: the permeation (absorption) rate, which will determine the C_{max} (and acute toxic effects), and the total amount systemically absorbed during a certain period (e.g. 24 hours).

This aspect may also be important when comparing the effects of acute and longer duration systemic exposures via inhalation and through the skin. Even assuming identical fractional uptakes via the two routes, the dermal uptake will generally be slower, but it may continue longer due to the lag time and the presence of a skin reservoir (especially for lipophilic compounds).

For investigating the various problems in the application of QSPeR results, mechanistically based mathematical models for dermal permeation may present a helpful tool, although one must be aware of the limitations of their use. Further, in the interpretation, extrapolation and application of experimentally obtained dermal absorption data, mechanistic models describing the dermal permeation process may prove to be useful, especially for proper retrieval of descriptive parameters such as lag time and permeability coefficient, extrapolation between infinite and finite doses, and the simulation of the permeation after occupationally relevant exposure scenarios (e.g. short duration, intermittent, repeated exposures) based on limited experimental data.

For neat solvents and possibly also for non-aqueous mixtures of poorly water-soluble, lipophilic compounds the K_p's derived from experimental flux data can probably not easily be compared with QSPeR predicted K_p's, which are based on data obtained with aqueous solutions, since the actual driving force for permeation is based on the activity and not on the concentration.

In that case thermodynamic arguments (activity, water solubility) may help to extrapolate from aqueous to non-aqueous exposure conditions. In this regard, it is the concept of maximum flux that defines the highest dermal exposure risk for a chemical, and QSARs based on this approach (Magnusson *et al*., 2004a) may offer a better approach to dermal risk assessment.

Since neat liquids and saturated solutions will have the same (maximum) thermodynamic activity, the dermal flux should be the same for neat liquids and saturated solutions, provided the vehicle or the neat chemical does not affect the barrier properties of the skin (CEFIC, 2004; Frasch, 2007). The maximum dermal flux of a chemical should thus occur at its solubility limit, and can therefore be estimated by multiplying its permeability coefficient obtained in water by its water solubilty (WHO, 2006). This approach may be especially useful for petroleum products and especially neat petroelum hydrocarbons.

In the case of mixtures, the maximum flux of each constituent may be considered as a very worst case approach, since we are not dealing with neat solutions but compounds that represent only a fraction of the solution mixture.

If the outcomes still pose a problem, the calculated (maximum flux) may be corrected by a factor representing the fractional amount of the compound in the solution. In this approach the difference in lipophilicity between the neat compound and the mixture (determining the partitioning of the compound into the skin) will be ignored.

It may be concluded that whilst the concept of maximum flux may seem to be rather attractive, in practice application of this will be quite complex, especially for the limited concentrations of individual compounds in (complex) mixtures.

A serious problem in the development and use of mathematical models is the effect of the permeant or the vehicle on the skin barrier properties. For solvents prolonged contact with the skin may result in penetration into the skin and thereby gradually change both the structure and the physicochemical properties of the skin. Contact with highly lipophilic solvents or permeants may even result in damage or destruction of the skin barrier. The time course of these effects is difficult to predict and to model in a quantitative way. Moreover the effects may be related to individual susceptibility. This will seriously hamper extrapolation of dermal permeation data between short and prolongued exposure.

In spite of all the shortcomings in the methods for estimating dermal absorption, the presently available models (QSPeRs) can help in the categorisation of compounds (solutes) as good, bad or intermediate skin permeants (Magnusson *et al*., 2004b; WHO, 2006).

In the absence of detailed exposure data and information on the skin permeation properties, a more generic and conservative approach, like that proposed by De Heer *et al*. (1999), may be followed to arrive at a worst case estimate of the 'safe' exposure level.

2.2.7. Comparison of experimental data on dermal absorption of petroleum hydrocarbons with model predictions

One of the main obstacles to wider application of predictive modelling for dermal absorption is their limited evaluation and validation with experimental data. In this section, the experimental data on percutaneous absorption of petroleum hydrocarbons presented in Chapter 4 are compared with the values predicted using the SKINPERM and DERMWIN models. The data on physico-chemical properties needed for modelling and the predicted values are shown in **Tables I** and **II**. As illustrated in **Figure 4**, the experimental K_p is inversely proportional to the lipophilicity of the petroleum hydrocarbons (expressed as log K_{ow}), which is in contrast to the trend obtained from both prediction models. The predicted K_p values were similar for both models and showed a significant overprediction when compared with experimentally derived K_p values obtained in studies performed with petroleum products or neat solvents. There is a better agreement between predicted and experimental values for water solutions, which is to be expected since both models are based on studies with aqueous solutions (**Figure 4**, closed circles). However, for the maximum flux, which is calculated using K_p values predicted by the DERMWIN model ($Flux_{max}$= K_p x water solubility), a decrease in maximum flux with increasing K_{ow} is obtained. This is consistent with the trend obtained in experimental studies (**Figure 5**). Interestingly, six of the data points clearly deviate from other values. These points represent the values of aromatic compounds with 3 or more rings (anthracene, phenanthrene, fluorine, pyrene, chrysene and benzo[a]pyrene), which are characterised by very low water solubility.

In conclusion, this result shows the advantage of using maximum flux instead of K_p in the risk assessment of highly lipophilic hydrocarbons.

Figure 4 Relationship between the K_p and log K_{ow}. The values represent the experimental data presented in Chapter 4 and the values predicted by the SKINPERM and DERMWIN models

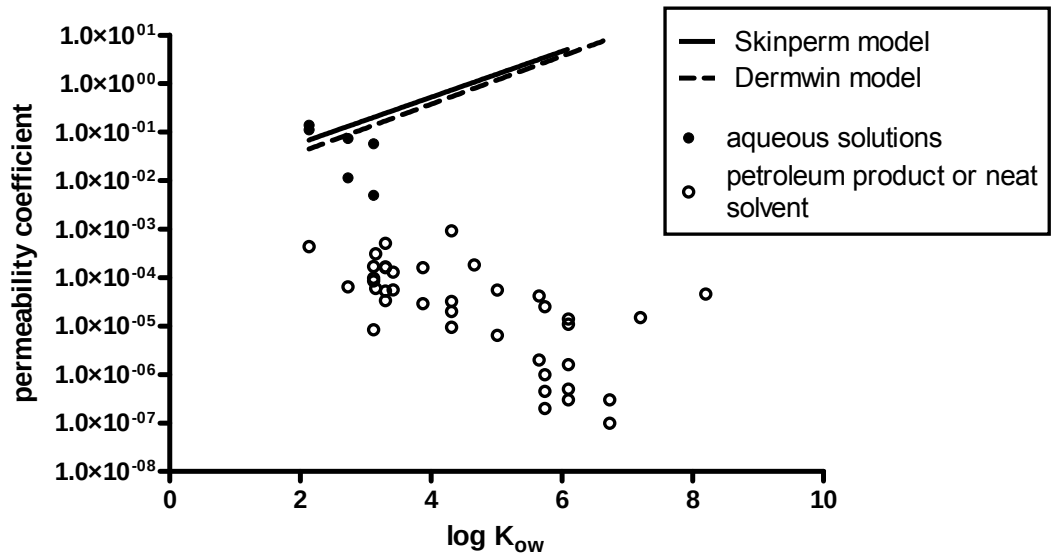


Figure 5 Relationship between the maximum dermal flux and log K_{ow} predicted by the DERMWIN model

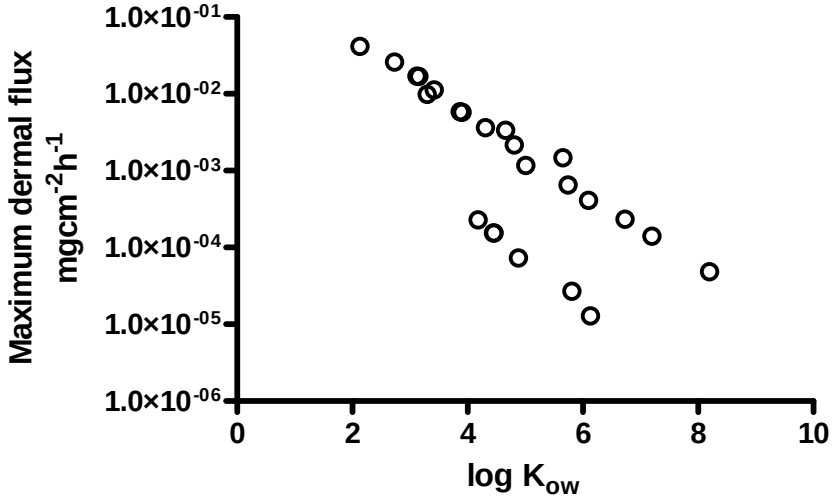


Table I Physico chemical properties of selected hydrocarbons used by the predictive models SKINPERM and DERMWIN

Chemical	CAS number	MW Da	Vapour pressure Pa (25 °C)	Water solubility mg/l	$logK_{ow}$ SKINPERM	$logK_{ow}$ DERMWIN
Hexane	110-54-3	86.20	20000	10	3.50	3.90
Heptane	142-82-5	100.20	6130	3.4	4.66	4.66
Nonane	111-84-2					5.65
Decane	124-18-5					5.01
Undecane	1120-21-4	156.31	55	0.257	5.74	5.74
Dodecane	112-40-3	170.34	18	0.0037	6.10	6.10
Tridecane	629-50-5					6.73
Tetradecane	629-59-4					7.20
Hexadecane	544-76-3					8.20
Benzene	71-43-2	78.00	12700	1780	2.13	2.13
Trimethylbenzene	25551-13-7					3.42
Ethylbenzene	100-41-4	106.17	1280	169	3.15	3.15
Toluene	108-88-3	92.14	3800	526	2.73	2.73
$xylene_{izomer\ mixture}$	1330-20-7	106.00	1330	200	3.00	3.12
Anthracene	120-12-7	178.24	0.06600	0.04340	4.45	4.45
benzo[*a*]pyrene	50-32-8	252.00	0.00000073	0.00162	6.13	6.13
Pyrene	129-00-0	202.00	0.0008	0.135	4.88	4.88
Chrysene	218-01-9					5.81
Fluorine	86-73-7					4.18
Phenanthrene	85-01-8					4.46
Naphthalene	91-20-3	128.00	4.0	30	3.37	3.30
methylnaphthalenes	1321-94-4					3.87
dimethylnaphthalenes	28804-88-8					4.31
trimethylnaphthalenes	28652-77-9					4.81

Table II Permeability coefficient (K_p) and maximum flux predicted by the SKINPERM and DERMWIN models

Chemical	K_p (cm/h) SKINPERM	K_p (cm/h) *DERMWIN*	$logK_{ow}$ SKINPERM	$logK_{ow}$ DERMWIN	*Maximum flux SKINPERM*	*Maximum flux DERMWIN*
Hexane	0.241	0.334	3.50	3.90	0.002410	0.00576
Heptane	1.37	0.949	4.66	4.66	0.004650	0.003370
Nonane		3.230		5.65		0.001480
Decane		0.931		5.01		0.001170
Undecane	3.49	2.540	5.74	5.74	0.000897	0.000653
Dodecane	5.13	3.730	6.10	6.10	0.000019	0.000410
Tridecane		8.520		6.73		0.000234
Tetradecane		15.200		7.20		0.000140
Hexadecane		52.500		8.20		0.000048
Benzene		0.0207	2.13	2.13	0.048800	0.041400
Trimethylbenzene	0.0274	0.0944		3.42		0.01130
Ethylbenzene		0.0739	3.15	3.15	0.016500	0.016900
Toluene	0.0978	0.0453	2.73	2.73	0.031600	0.026000
$Xylene_{iisomer\ mixture}$	0.0601	0.0704	3.00	3.12	0.0152	0.017100
Anthracene	0.106	0.225		4.45		0.000155
Benzo[*a*]pyrene		1.240		6.13		0.000013
Pyrene	0.281	0.324		4.88		0.000073
Chrysene	1.49	1.030	4.45	5.81	0.000012	0.000027
Fluorine	0.399	0.171	6.13	4.18	0.000002	0.000229
Phenanthrene		0.229	4.88	4.46	0.000054	0.000155
Naphthalene		0.0694		3.30		0.009860
Methylnaphthalenes		0.145		3.87		0.005890
Dimethylnaphthalenes	0.10	0.244		4.31		0.003620
Trimethylnaphthalenes		0.454	3.37	4.81	0.003010	0.002170

3. METHODS FOR THE ASSESSMENT OF DERMAL EXPOSURE

3.1. EXPERIMENTAL METHODS

3.1.1. Sampling methods

Contamination or exposure of the skin surface may occur by a number of mechanisms. Substances can be deposited on the skin directly from the air or due to contact of the skin with the contaminated surface. It may also arise from intentional or accidental immersion into the liquid or powder or due to spillage onto the skin during handling. Additionally, the processes such as evaporation of the contaminant from the skin or uptake of a contaminant through the skin make the evaluation and quantification of skin exposure even more complex (Schneider *et al*., 1999).

The methods for the assessment of dermal exposure can generally be divided into three groups: removal methods, collection (surrogate skin) methods and fluorescent tracer techniques. Despite the existence of several comprehensive guidelines (HSE, 1999; OECD, 1997; US-EPA, 1986; WHO, 1982) the main problem with application of the methods for measuring skin exposure is that they are not yet properly standardized nor validated due to (1) lack of consensus regarding the differences in the underlying assumptions for the various methods, (2) lack of emphasis on the development of a basic model of dermal exposure and (3) lack of consistent terminology (Schneider *et al*., 1999). This also hampers the comparison of results obtained using the various methods.

3.1.2. Removal methods

Removal methods are widely used and their advantage lies in the low cost and ease of application. In this technique, a substance deposited on the skin may be removed chemically (hand washing and rinsing) or mechanically (skin wiping, tape stripping) and subsequently determined by appropriate analytical method.

Hand washing/rinsing and wiping

Hand washing and wiping are common methods for the assessment of dermal exposure because of their accessibility and ease of use. The methods have been reviewed by Brouwer *et al*. (2000a). The washing method is based on washing or rinsing the hands in a bag/bottle containing an appropriate solution of solvent or detergent in a routine washing procedure for a certain period of time at the end of exposure. The amount of solution should be adequate for sampling the entire hands. In this way the chemical is removed from the surface of the skin, with the solvent or detergent acting to enhance removal (Tucker *et al*., 2001; Brouwer *et al*., 1998; Fenske *et al*., 1999). The hand wiping method is based on removal of the contaminant from the surface of the skin (usually limited by a template) with wipes usually soaked in solvent, water or mixtures that are not damaging to the skin. While the hand washing method allows exposure of the whole hands to be estimated (expressed in units of mass per body part), hand wiping samples discrete/limited surface areas enabling expression of the mass removed from the skin in terms of mass per surface area e.g. $\mu g/cm^2$.

To assess the contamination level quantitatively, the sampling efficiency must be known. For that purpose two approaches have been used: mass balance and direct

spiking (Fenske and Lu, 1994; Fenske *et al*., 1998; Brouwer *et al.*, 2000a) whereby the first approach mimics the transfer of a non-liquid contaminant and the latter the transfer of a liquid contaminant. The sampling efficiency, defined as the percentage of a contaminant removed from the skin, depends on several parameters such as time of residence, level of skin loading, method of contamination, skin condition, solvent type, and number of consecutive washings or wiping, etc. For example, time of residence of a substance on the skin is an important variable for contaminants that penetrate the skin rapidly or bind to skin components which can result in a reduced recovery and an underestimation of the actual exposure. This has been demonstrated for chlorpyrifos and captan where prolonged residence times resulted in decreased sampling efficiency (Fenske and Lu, 1994; Fenske *et al*., 1998).

Fenske *et al*. (1999) compared rates of exposure of the hand by hand washing and hand wiping sampling method. They observed on average a 6 fold difference between the two methods, with hand wiping being lower. Incomplete removal by hand wiping was also demonstrated by McCurdy *et al*. (1994) and Fogh *et al*. (1999).

Tape stripping

Tape stripping is a non-invasive sampling technique used to assess dermal exposure. Generally, after the end of exposure, one or multiple layers of the uppermost layer of the skin, the s*tratum corneum*, are removed by adhesive tape and analyzed for the chemical. The method has been applied to study the dermal exposure to multifunctional acrylates (Surakka *et al*., 1999; Nylander-French, 2000), metals (Cullander *et al*., 2000) and jet fuel (Mattorano *et al*., 2004; Chao *et al*., 2005). It was suggested that the washing technique could be a more accurate method than the tape stripping technique due to the variation caused by the operator performing the sampling (Roff *et al*., 2001; see also chapter *in vivo* methods). Mattorano *et al*. (2004) showed, in accordance with the results obtained by another technique (Fenske and Lu, 1994; Fenske *et al*., 1998), that the residence time in the skin dramatically influences the sampling efficiency for naphthalene. For jet fuel increasing the time of residence from 5 to 20 minutes resulted in a marked decrease from 69.8% to only 0.9%. According to the authors this indicates that jet fuel rapidly penetrates through the *stratum corneum*.

3.1.3. Surface collection methods

Collection methods, also called surrogate skin techniques, refer to placing a collection medium on the skin surface or clothes for a certain period of time and its subsequent analysis for chemical content. The methods include patch sampling, whole body dosimetry and glove collection. The main assumption is that the collection medium/item captures and retains the chemicals in the same way as the skin but this is not necessarily the case since they do not have same properties as the skin. Another limitation of this technique is that it measures mass of the chemical deposited on the skin and not the concentration (e.g. mg/ml) which is the driving force for diffusion of the chemical through the skin (Cherrie and Robertson, 1995).

Patch sampling and whole body dosimetry

Patch collection and whole body dosimetry techniques have been reviewed by Soutar *et al*. (2000). Both methods measure the amount of a contaminant on the skin surface expressed per unit area. Typically, the dimension of the patches is approximately 10 cm x 10 cm although smaller patches have also been used. The area monitored represents around 3-8% of the body surface depending of the

number of patches used. The exposure is estimated by extrapolation of the patch area to the body surface. Another assumption which may lead to errors is that the patches represent the body surface contamination and hence that exposure is uniform over the whole body surface area. The various protocols use different standard body surface areas (OECD, 1997; US-EPA, 1986; WHO, 1982). The main drawback of the estimation of exposure is that extrapolation can lead to under- or over-estimation, errors being larger for smaller patches. Tannahill *et al*. (1996) observed differences of more than two orders of magnitude in a comparison of patches and oversuits. Whole body dosimetry, which measures the contaminant deposited on clothing overcomes this problem since the whole area is represented and no scaling is required. Additionally, it does not rely on an assumption that the distribution of the contaminant is uniform. However, a disadvantage is the need to use large volumes of extracting solvent resulting in only a small concentration of the contaminant which might present a problem for chemical analysis.

An additional problem is the use of different materials as a collection media, such as cotton, polypropylene, polyester, flannel and cellulose paper which hampers good comparison of studies. Care should be taken to prevent the saturation of the patches or garment. An assessment of the sampling efficiency should be performed prior to any field survey, as in case for removal techniques.

Sampling of volatile substances presents a problem in exposure measurement. In an attempt to overcome this problem, Cherrie and Robertson (1995) proposed the usage of a patch sampler that will collect the contaminant by diffusion. The sampler, comprising an adsorbent and semi-permeable membrane, should mimic the process of uptake through the skin thereby providing estimates of exposure that are “biologically relevant”.

Vacuuming techniques, mostly used for sampling from contaminated surfaces other than skin, have been regarded as poor collection technique due to their low removal efficiency (Byrne, 2000; Glass *et al*., 2003). In a study by Lundgren *et al*. (2006) on the dust deposition of cornstarch and wheat flower on the skin three methods were compared: vacuuming sampling, tape stripping and patch sampling. They reported good agreement between vacuum sampling and tape stripping and slight overestimation for patch sampling compared to tape stripping. This was explained by particles remaining stuck on the glue.

Glove collection

Absorbent gloves can be used to collect the contaminant by replacing or covering the protective gloves or placement under protective gloves. They are easily used, especially in field conditions, and they efficiently absorb the contaminant, which will otherwise be absorbed into the skin. Still, it is reported that gloves usually overestimate the exposure compared to, for example, hand washing and wiping (Fenske *et al*., 1999). Gloves should not become saturated, and to avoid this, it is recommended to regularly replace them (OECD, 1997). The method cannot be used for reliable estimation of whole body exposure due to the non-uniform distribution of contaminant on the skin and the large extrapolation factors needed.

3.1.4. Other procedures

Fluorescent tracer method

The fluorescence tracer technique is based on the inherent property of a substance to emit visible light when illuminated with ultraviolet radiation. It has been used to

assess surface deposition of naturally fluorescing substances, like polycyclic aromatic hydrocarbons (Ness, 1994). Since the procedure can easily locate the contamination, it enables the demonstration of non-uniform exposure, which contributes substantially to the uncertainty around the level of dermal exposure and finally risk assessment (Fenske *et al*., 1990). Combination of the fluorescent tracer technique with image processing (Video Imaging Technique to Assess Exposure, VITAE) provides a quantitative method which enables simultaneous assessment of the amount of a contaminant on the surface of skin and the area of exposed skin (Cherrie *et al*., 2000). The method has also been used to assess the efficiency of protective garments (Fenske *et al*., 2002). Semi-quantitative methods using a visual score system, which are based on visual observation and scoring of both the extent and intensity of the fluorescent tracer on the skin are also available (Fenske *et al*., 1988; Aragon *et al*., 2006).

Since the number of naturally fluorescing substances is relatively small, it is common to add a fluorescing substance as a tracer to follow the dispersion of a contaminant. The disadvantage of such an approach lies in the assumption that the added fluorescent tracer shows the same pattern of deposition, dispersion and retention characteristics as the contaminant. This method ignores the loss of contaminant from the surface of the skin by evaporation or by washing. Additionally, the fluorescent tracer can also bind to the skin which makes it less easily removed (Cherrie *et al*., 2000). The results of a fluorescence tracer study have been compared to those using chemical analysis; in the first study (analysis of coveralls) a large variation was reported between methods, while in the second study (washing of the skin) the correlation was good (Roff *et al*., 1997; Brouwer *et al*., 2000b). In both studies it was concluded that at low concentration exposure levels the fluorescence tracer is not as good as chemical analysis. Roff *et al*. (2001) also compared the fluorescence tracer method with the rinsing method and showed good agreement between these two completely different methods. In a very recent study, dermal exposure to semi-synthetic metal working fluids using a surrogate skin method (pads) and VITAE was measured (van Wendel de Joode *et al*., 2007). The study showed that dermal exposure levels estimated by VITAE were lower, by a factor of 3, when compared to pads.

Conceptual model

It has been suggested that a conceptual model proposed by Schneider *et al*. (1999) to assess dermal exposure could act as a starting point for the development of a sampling strategy and form the basis for improved comparability between studies (Vermeulen *et al*., 2000; Soutar *et al*., 2000). The model describes exposure as the result of mass transport between six identified compartments where the hazardous substance may reside (source, air, surface contaminant layer, outer and inner clothing contaminant layer and skin contaminant layer). The proposed assessment/measurement strategy is based on a tiered approach which follows the protocol presented in European Standard EN 689 (CEN, 1995). In a first step, all sources of potential exposure should be identified by preparation of lists of all chemical substances present in the workplace with their corresponding toxicological information. In the second tier an evaluation of workplace factors including tasks, work patterns and techniques, production processes, sources of direct contact, spilling, splashing and emission to air, safety precautions and procedures should take place. The third tier should consist of a structured approach to assess the exposure. If dermal uptake cannot be ruled out a basic survey should be made with the purpose of providing quantitative information about the distribution and level of dermal exposure (Schneider *et al*., 2000). The ideal basic survey would consist of a dermal uptake monitor with corresponding dermal occupational exposure limits (DOEL). Different approaches to develop DOEL values for occupational practice

have been suggested, however, no consensus has yet been reached concerning establishment of these values (Bos *et al*., 1998; Nielsen & Grandjean, 2004).

3.1.5. Dermal exposure measurement of petroleum hydrocarbons

There are very few dermal exposure studies concerning petroleum hydrocarbons. Van Wendel de Joode *et al*. (2005) evaluated charcoal cloth pads for the assessment of dermal exposure to benzene and toluene in workers performing four different jobs in a petrochemical plant. Activated charcoal pads with an area of 4 cm x 3 cm were worn on the wrist of the hand of preference during a full-shift of 8 hours. The determined recoveries of charcoal pads ranged from 85 to 100% for both benzene and toluene. The average amount of benzene deposited on the pad was between 0.46 and 2.37 μg/cm^2/8h and for toluene was between 0.69 and 11.99 μg/cm^2/8h. Mattorano *et al.* (2004) investigated dermal exposure to neat jet fuel using an adhesive tape stripping technique. The subjects (n = 22) were exposed on the ventral surface of each arm (exposure area 10 cm^2) for 5, 10, 15 and 20 minutes. After the end of exposure, adhesive tapes were applied on the exposed site. Three consecutive strips were taken. As a marker of exposure naphthalene was determined in the tape strips. On average the first tape strips taken 5 minutes after exposure removed 21.6 μg/cm^2 of naphthalene. The amount recovered with first tape strip decreased to 0.24 μg/cm^2 after 20 minutes of exposure. In the second tape strip the 0.15 μg/cm^2 of naphthalene was removed after 5 minutes decreasing to 0.022 μg/cm^2 after 20 minutes of exposure. The data indicate that naphthalene penetrates rapidly into the deeper layers of the *stratum corneum*. In the second study of the same group (Chao *et al*., 2005) dermal exposure of a USAF fuel-cell worker (n = 124) to jet fuel performing different jobs was investigated. Exposure was assessed by using tape stripping technique and naphthalene was measured in tape strips as a marker of exposure. The subjects were monitored using several body regions with the greatest potential for exposure: forehead, neck, shoulders, arms, hands, legs, knees, feet and buttocks. Three successive tape strips were taken from each body region. Overall, dermal exposure to naphthalene was different among the sampled regions with the highest being on the buttocks of one subject. The authors calculated a whole body exposure to naphthalene of 2.02 μg/m^2. Significant differences were observed between the high-exposure group (4.19 μg/m^2) and the low-exposure group (0.34 μg/m^2), while there was no significant difference between the low-exposure and the medium-exposure (0.48 μg/m^2) groups.

3.1.6. Summary of the experimental methods

Various methods have been developed and used for measuring dermal exposure. In essence, two main approaches can be distinguished: removal methods and collection methods. The various aspects of these approaches have been described and discussed in this chapter, and some specific examples for petroleum hydrocarbons have been presented. Additionally methods using fluorescent tracers were discussed and compared with other exposure assessment methods. The fluorescent tracer method has been used to demonstrate the non-uniformity of exposure, and to assess the efficiency of protective garments. The technique may be especially useful for petroleum products containing naturally fluorescing (polycyclic) aromatic hydrocarbons.

The methods and results may be applied in two ways.

1. For monitoring dermal exposure during specific occupational situations and/or when handling specific product types

2. To create a database of exposure data that can be used in creating and validating more generalized approaches (exposure models) for the assessment of dermal exposure.

The first application will especially be aimed at maintaining and/or improving measures for keeping dermal exposure at a level generally considered to be safe (occupational hygiene).

In the second application, exposure models can be developed for the assessment of dermal exposure under various conditions. Provided they are properly validated, these models may then be used reliably in risk assesssment procedures for dermal exposure (combined with assessment procedures for dermal absorption as appropriate).

In practice it turns out that current methods for measuring skin exposure are, for several reasons, not yet properly standardized nor validated (Schneider *et al*., 1999). This will hamper both comparison of results obtained by the various methods and an objective evaluation of results in the context of risk assessment.

For petroleum hydrocarbons, the scope of occupational activities in the petroleum industry and the size of the database needed to enable the development and validation of exposure models may turn out to be too limited at this moment. In particular, the specific physicochemical properties that must be taken into account when designing and applying techniques for measuring exposures. In this respect it is important to recognise that petroleum products are (generally) characterized by a high lipophilicity, are often of high volatility, and the presence of different hazardous compounds (both aliphatic and aromatic). Also the exposure data that are already available must be critically evaluated with respect to the specific properties of the petroleum products in relation to the measuring techniques and materials used. In this respect, the use of specific compounds (e.g. naphthalene) in complex mixtures as markers for the total exposure (or absorption) should be viewed very critically, because of the possible differences (e.g. volatility, lipophilicity) with other compounds in the complex hydrocarbon mixture.

Finally, it must be bourne in mind that dermal exposure data may eventually be used to assess the amount that is systemically absorbed via the skin. In this respect it will be important to know not only the total amount deposited on the skin, but also the physical state (solid / liquid, neat / solution, type of vehicle / solvent), its concentration in the matrix (solution), the skin area exposed, the volume per surface area, the duration of exposure, the rate of flux through the skin and also the (various) anatomical sites of exposure.

3.2. MODELS FOR THE PREDICTION OF DERMAL EXPOSURE

Models have been developed for the assessment of occupational exposure particularly in cases when no measurements or very few data are available. There are only a limited number of models existing for dermal exposure e.g. EASE (ECB, 1996), EUROPOEM (EUROPOEM, 1996), RISKOFDERM (EU RISKOFDERM project, 5th framework program, project QLK4-CT-1999-01107), and DREAM (van Wendel-de Joode *et al*., 2003). Although the validity of these models has not been extensively studied (Marquart *et al*., 2003), in recent years some studies aiming at validating these models have been published (Hughson and Cherrie, 2001; van Wendel de Joode *et al*., 2005a,b).

3.2.1. Estimation and assessment of substance exposure (EASE)

EASE is a general model for prediction of occupational exposure applicable to a wide range of substances. It was originally developed by the UK Health and Safety Executive (HSE, 1996) for both dermal and inhalation exposure assessment of new chemicals prior to their introduction to the market. The model has been also used for purposes not originally envisaged: e.g. it is used to fill data gaps present in exposure measurement of existing substances and applied for regulatory risk assessment (Creely *et al*., 2005, Cherrie *et al*., 2003). At the time of writing this report, the EASE model 2.0 for dermal exposure was available and a prototype version of model 3.0 was under construction (Tickner *et al*., 2005).

The structure of this model is based on a series of basic criteria, adapted from an inhalation exposure model, representing the overall situation in the workplace: physical state, pattern of use and control, where the latter two parameters are given in a simple form, due to lack of reliable data on dermal exposure. A more detailed discussion of these basic criteria is available in a comprehensive report on EASE model 2.0 by Cherrie *et al*. (2003). The model predicts the potential exposure of hands and forearms only and is expressed as a mass per unit area exposed per day (e.g. mg cm^{-2} day^{-1}), since it was assumed that that hands and forearms would be the most commonly exposed body parts and this represents a skin area of about 2000 cm^2 (Tickner *et al*., 2005).

Although there were a number of published validation studies for the inhalation part of EASE (e.g. Bredendiek-Kämper *et al*., 2001; Hughson and Cherrie, 2005; Devillers *et al*., 1997; Hughson and Cherrie, 2000; Llewellyn *et al*., 2001, Mäkinen *et al*., 2002), there is only one validation study published for the dermal part (Hughson and Cherrie, 2001). Although this study seems less relevant in the context of this report because of the type of material investigated (zinc compounds), it is included here as a validation result of EASE for the dermal route. Hughson and Cherrie (2001) investigated the workplace dermal zinc exposure and compared it to the values obtained by the EASE model. Measurements were performed on subjects that were producing or working with zinc oxide, zinc powder and zinc dust in different workplaces. Dermal exposure was estimated by collecting dust from the different parts of the body with wet wipes (forearm, hands, forehead and chest) but only exposure data for hands and forearms were compared to values predicted by EASE. Furthermore, the tasks carried out in the factories were categorized in terms of the following three different EASE endpoints: non-dispersive use with intermittent direct handling, wide dispersive use with intermittent direct handling and wide dispersive use with extensive direct handling. The survey was divided into phases according to the sampling procedure: in the Phase 1 only the back of the hands were sampled and in Phase 2 both the palms and the back of the hands were sampled. The measured levels of zinc exposure for all categories and both phases ranged from 6 to 439 µg cm^{-2}. When the exposure was predicted by EASE it ranged from 100-15000 µg cm^{-2}, which clearly overestimated the dermal exposure by a factor of about 50. The interesting point is that the EASE predictions increased in line with the average measured exposures, which is similar to the validations of EASE performed for inhalation exposure scenarios (Devillers *et al*., 1997; Cherrie and Hughson, 2005). There are no validation data that show an agreement of EASE predictions with experimental/measured data on dermal exposure for liquids and non-metallic solids (Cherrie *et al*., 2003). The main limitation of the EASE model is that it only gives estimates of dermal exposure for hands and forearms, disregarding the level of dermal exposure for other areas of the body. The model also ignores the influence of hand/forearm washing, evaporation of contaminant from the skin as well as use of protective equipment (Tickner *et al*., 2005).

3.2.2. Dermal Exposure Assessment Method (DREAM)

DREAM is a model to evaluate occupational dermal exposure in a semi-quantitative manner (Wendel-de Joode *et al*., 2003) The aim was to provide an initial assessment of dermal exposure to liquids as well as solids, by estimating the potential and actual exposure, to give insight into the dermal exposure distribution over different body parts and finally to identify exposure routes. Additionally, by including job and task ranking, it provides information on potential measurement strategies and helps in determining what, where and who to measure (van Wendel de Joode *et al*., 2003). It is largely based on a conceptual model developed by Schneider *et al*. (1999).

A DREAM assessment consists of two parts: the inventory part addresses general information and possible dermal exposure determinants, as identified in the conceptual model of Schneider *et al*. (1999). It consists of six modules (company, department, agent, job, task and exposure) structured hierarchically in the form of a questionnaire to be filled by an occupational health professional. The evaluation part determines the potential dermal exposure (i.e. the predicted contamination of clothing and uncovered skin due to three different (consecutive) exposure routes - emission, transfer and deposition) and actual dermal exposure (i.e. the exposure on skin) for nine body parts. It is organized in such a way that each estimate is determined by a set of underlying variables, from a total of 33 variables. According to the authors, the advantage of DREAM lies in its ability to document the decisions made by the investigator in a structured way (van Wendel-de Joode *et al*., 2003). In order to assess the reliability of the model the authors of DREAM conducted a series of studies on dermal exposure where inter-observer agreement, the effect of the individual observer on dermal exposure estimates for different tasks and comparison of inter-observer agreement for ranking of body parts according to their exposure level were assessed (van Wendel-de Joode *et al*., 2005a). The study showed good reproducibiliy for inter-observer agreement, covering a broad range of tasks comprising exposure to liquids, solids and vapours. Furthermore, to assess the accuracy of their method they compared DREAM estimates with quantitative dermal exposure measurements in six occupational settings (van Wendel-de Joode *et al*., 2005 b). The results showed that the accuracy of DREAM estimates varied within and between surveys. The limitation of the study lies in the fact that it was performed only for liquids. Another limitation is the inability to verify the accurate assessment of spatial variability. This was due to the lack of sufficient measurements on body parts other than hands.

3.2.3. RISKOFDERM

RISKOFDERM is an EU funded project which aims at (1) developing a toolkit for the assessment of dermal exposure in small and medium-sized enterprises and (2) development of dermal exposure models for regulatory risk assessment (Warren *et al*., 2003, 2006, van Hemmen, 2004; van Hemmen *et al*., 2003; Marquart *et al*., 2006). A series of papers have been published presenting the main framework of the toolkit (Goede *et al*., 2003; Marquart *et al*., 2003; Oppl *et al*., 2003; Warren *et al*., 2003). The toolkit is based on theoretical approaches for the following determinants: processes and tasks, substance and product characteristics, and situations and conditions. It assumes that operator exposure is influenced mainly by the tasks (Warren *et al*., 2003) for which six categories or DEO (dermal exposure operation) units are distinguished. Each of these categories has several modifiers divided into three groups (substance-specific modifiers, workplace–related modifiers and control measures) and to each group separate scaling factors are assigned

representing three different mechanisms of dermal exposure (direct contact, surface contact and deposition from an aerosol); this is explained in detail in the conceptual model by Schneider *et al*. (1999). This division of groups will lead to derivation of default exposure values for each task, which can be revised as new quantitative data become available on dermal exposure (Marquart *et al*., 2006). Development of dermal exposure models for regulatory risk assessment applies two approaches; a first is derivation of default values for each of six designated DEO-s (Marquart *et al*., 2006) and the second is a model based on statistical relationships between measured data and potential exposure determinants for each of the six DEO-s (Warren *et al*., 2006). Although, default values derived for potential dermal exposure of the hands are considered useful for similar scenarios and substances used, the uncertainty in the assessment using those values cannot be quantified (Marquart *et al*., 2006), The aim of the project was to design a model for each of the dermal exposure situations, but this is not yet possible due to insufficient reliable and precise data (Warren *et al*., 2006).

3.2.4. European predictive operator exposure model (EUROPOEM)

EUROPOEM is a harmonized, predictive model for operator exposure during application, based on the results of field studies. It is used only in the registration process for agricultural pesticides in the European Union. The predictions obtained from this model are compared to acceptable operator exposure level (AOEL, derived from relevant toxicological data) to assess whether the recommended application technique for the proposed pesticide is safe. When the ratio of exposure and AOEL is less then 1 the exposure scenario is considered acceptable. In the case of exposure and AOEL ratios exceeding 1, according to the tiered approach, this will lead to a more-detailed exposure assessment. The database was developed using only those studies performed according to the OECD guidance document (1997) unless they have been done before the Guidance document came into effect. Those data were also evaluated in order to have a representative set of data in the database. The exposure data can be calculated and presented either as the amount of active substance handled, or as the amount of formulation or spray volume per unit of time (van Hemmen, 2001).

3.2.5. Summary of models for prediction of dermal exposure and their applicability to petroleum products

In this chapter various models for the prediction of dermal exposure have been described and discussed namely EASE, DREAM, RISKOFDERM and EUROPOEM.

Although many efforts have been made in the development and optimization of these models, their specific application for the estimation of dermal exposure to petroleum hydrocarbons seems to be limited.

Schneider *et al*. (1999) proposed a conceptual model, that has been used in the development of the DREAM and RISKOFDERM models. At this time the practical application of these models for petroleum products in occupational settings has not been widely practiced. Further development and validation directed to these types of products and industries is needed.

Although EASE is a widely applied model for the prediction of occupational exposure, application of this model for dermal exposure asssessment of petroleum products is severely hampered by the lack of relevant validation data. There is only one validation study available for the dermal exposure part, but this concerns zinc

compounds (zinc oxide, zinc powder and zinc dust), which are not considered to be directly relevant to petroleum products. A further draw back is the fact that the EASE model provides only estimates of dermal exposure for hands and forearms and does not take into consideration the effects of hand/forearm washing, evaporation from the skin and the use of protective equipment. In particular evaporation from the skin may be an important factor for certain (volatile) compounds in petroleum products.

EUROPOEM is a data-based model for the prediction of operator exposure during mixing and application of agricultural pesticides. Therefore it can be concluded that application in petroleum industries is unlikely to be useful and hence it is not considered further.

Overall it is concluded that the available models for prediction of dermal exposure are not (yet) useful for providing a reasonably accurate dermal exposure prediction for pretroleum products. Therefore one should either use a worst case approach or perform actual occupational exposure studies and/or invest in further development and validation of the models presented above.

4. LITERATURE STUDIES ON DERMAL ABSORPTION OF PETROLEUM HYDROCARBONS

4.1. INTRODUCTION

The purpose of this brief review is to summarize relevant data on the dermal absorption of petroleum hydrocarbon products and their constituents as well as a critical evaluation of the available data. The data taken into consideration cover various aliphatic and aromatic hydrocarbons present in petroleum products that may come in contact with skin, either as individual chemicals or as part of the mixtures and commercial products.

4.2. PROCEDURE

To achieve the objective the following four steps were performed.

Step 1:

Identification of relevant petroleum hydrocarbons and available literature data.

Aliphatic hydrocarbons	Aromatic hydrocarbons
- Hexane	- Benzene
- Heptane	- Toluene
- Nonane	- Xylene
- Decane	- Methylbenzene, ethylbenzene, trimethylbenzene
- Undecane	- Naphthalene
- Dodecane	- Methylnaphthalene, dimethylnaphthalene, trimethylnaphthalene, tetramethylnaphthalene
- Tridecane	- Pyrene and benzo[a]pyrene
- Tetradecane	

Step 2:

A searching of scientific bibliographic databases to identify publications that were not covered in a previous CONCAWE report on dermal absorption of petroleum hydrocarbons (Petroleum hydrocarbons: their absorption through and effects on the skin, CONCAWE, 1984).

Databases and web pages searched were:

www.pubmed.com

www.scholargoogle.com

www.google.com

Keywords used for the search of databases were:

“Name of the pure chemical” + percutanoeus absorption

“Name of the pure chemical” + percutaneous penetration

“Name of the pure chemical” + dermal absorption

“Name of the pure chemical” + dermal penetration

"Name of the pure chemical" + dermal permeation

"Name of the pure chemical" + skin absorption

"Name of the pure chemical" + skin penetration

"Name of the pure chemical" + skin permeation

Additionally, instead of "Name of the pure chemical" the following keywords were used: petroleum, petroleum hydrocarbons, kerosene, jet fuel, coal tar (although not a petroleum product), gasoline, aliphatic hydrocarbons, aromatic hydrocarbons and PAHs or polycyclic aromatic hydrocarbons.

The search was also performed by replacing "Name of the pure chemical" with CAS numbers of pure chemicals.

Step 3:

A critical evaluation of the available data.

Questions addressed were:

i. Is the exposure performed to pure chemicals or to a mixtures or commercial products?

ii. Are the data and the manner in which they were obtained scientifically valid?

iii. For better comparison all the parameters were transformed into uniform units (i.e. K_p in cm/h, flux in mg/cm^2/h).

Step 4:

The analyzed data were summarized in standardized tabular formats (per study and per compound) to enable an effective use of the available information.

4.3. OVERVIEW OF THE STUDIES ON DERMAL ABSORPTION OF HYDROCARBONS

In the following tables, an overview has been given of the experimental studies on dermal absorption of petroleum hydrocarbons.

In **Tables 1-20** listed in **Appendix 1 of this report** summary data on dermal absorption parameters for individual hydrocarbons have been presented.

In a second set of tables (**Tables 1-49** listed in **Appendix 2 of this report**) a more detailed description of experimental conditions and interpretation of the outcomes is presented.

4.4. SUMMARY OF LITERATURE STUDIES ON DERMAL ABSORPTION OF PETROLEUM HYDROCARBONS

- There is substantial evidence from both human and animal *in vitro* as well as *in vivo* studies that aliphatic and aromatic compounds reviewed in this report are able to penetrate and permeate the skin when applied as pure substances, in mixtures or as commercial products.

- Generally, higher absorption of aromatics (e.g. toluene, xylene, naphthalene) than aliphatics (decane, dodecane, tridecane and hexadecane) has been reported.
- Aliphatic hydrocarbons show higher tendency to remain in the skin which might have influence on local effects such as irritation.
- Presented data on dermal absorption of individual hydrocarbons from petroleum products such as jet fuel, strongly suggests that dermal exposure to these products would not cause systemic toxicity under normal working conditions and assuming an intact skin barrier.
- Only 6 of 49 dermal absorption studies described in this report were done with chemical vapours and these were all done *in vivo*. These studies revealed the limited significance of dermal exposure to vapours of hexane, benzene, toluene, and xylene. The absorption of vaporous hydrocarbons is negligible in comparison to that of liquid hydrocarbons. Even when whole-body dermal exposure is considered, dermal uptake contributes only a few percent of the total (inhalatory + dermal) body burden.
- For some hydrocarbons, including benzene, toluene and xylene, the absorption from aqueous solutions was several orders of magnitude higher that the absorption after dermal exposure to either a neat chemical or a petroleum product e.g. jet fuel. This emphasises the need for conducting absorption studies under ''in use'' scenarios. In the application of QSPeRs (K_p) it should be kept in mind that for their development permeability coefficients are used that are obtained with aqueous vehicles.
- A few comparative studies showed that rat skin is more permeable than human skin. For toluene and xylene this difference was roughly 10-fold. Pig skin permeability was similar to that of human skin.
- Almost all experimental studies were performed in “normal” untreated skin and no data were found for compromised skin. In two studies skin was used that was treated with baby oil, moisturizer, insect repellant and sunscreen cream or jet fuel before exposing to the compounds.
- Most of the *in vitro* studies were performed with split thickness skin.
- Pre-exposure to jet fuel led to higher (2- to 4- fold) absorption of the aromatic and some aliphatic hydrocarbons implying alteration of the skin barrier probably due to lipid extraction from the *stratum corneum.*
- Dermal absorption can significantly be affected by viscosity of a petroleum product. In general, higher viscosity leads to reduce dermal absorption.

Some gaps in our knowledge were identified:

- The lack of dermal absorption studies in compromised skin.
- The need for information on the effect of repeated exposure on the dermal absorption of petroleum hydrocarbons.
- The effect of the vehicle composition (mixture, aqueous neat liquid) and additives on dermal absorption.
- The effect of the concentration of individual compounds in complex petroleum product on their permeability (and flux).

4.5. USE OF DERMAL ABSORPTION STUDIES IN RISK ASSESSMENT: EXAMPLE OF BENZENE

To illustrate the way in which dermal absorption data can be used for risk assessment the contribution of dermal uptake to the total uptake (inhalatory + dermal uptake) has been estimated for benzene. Benzene has been taken as an example for several reasons. First, unlike the other key constituents, benzene is classified as a human carcinogen.. Secondly, dermal flux of benzene is one of the highest among petroleum hydrocarbons and reliable experimental data on dermal absorption of benzene is available in the literature for both aqueous solutions and petroleum products. Furthermore, for benzene there is an occupational limit value for indoor air concentration enabling comparison of inhalatory and dermal uptake routes in an occupational context.

Dermal intake of benzene resulting from skin exposure to a petroleum product (gasoline) was estimated using experimental results based on the study of Adami *et al*. (2006).

The experimentally determined skin flux amounted to 1.99 µg cm^{-2} hr (K_p 43.8 cm hr 10^{-5})

The following exposure scenario has been assumed:

The concentration of benzene in gasoline is: 0.7 % (average of 3 gasoline samples).

Duration of skin exposure is: 60 minutes (cumulative exposure over an 8-hour shift).

The exposed skin area: 1000 cm^2.

Air concentration: 3.2 mg m^{-3} corresponding to the occupational exposure limit value in most countries at the time of writing this report. Furthermore, this value is expected to represent a “reasonable worst case” in the petroleum industry.

Assuming a respiration rate of 0.6 m^3 hr, the estimated inhalatory uptake of benzene would be:

0.6 m^3 hr x 8 h x 3.2 mg m^{-3} = 15.4 mg

Therefore dermal uptake based on experimental data would be:

1.99 µg cm^{-2} hr x 1000 cm^2 x 1 h = 1.99 mg

Dermal uptake/(dermal + inhalatory uptake) = 1.99/ 1.99+ 15.4 = 11.4 %

The comparison of dermal and inhalatory uptake shows that dermal exposure to petroleum products can lead to uptake of benzene. However, it has to be emphasized that the exposure scenario used in this example was rather unrealistic that is a 1-hr exposure over a skin area of 1000 cm^2 does not occur under normal working conditions. However, this exposure scenario has been proposed by ECETOC for assignement of the skin notation (ECETOC, 1993).

5. METHODS FOR THE ASSESSMENT OF LOCAL SKIN EFFECTS

Skin exposure to petroleum products can lead to a variety of skin reactions varying in intensity from skin dryness and skin irritation to irreversible skin changes such as skin tumours (Koschier, 1999, Nessel *et al*., 1999a,b; Ritchie *et al*., 2003; Jia *et al*., 2002). Also individual aliphatic and aromatic hydrocarbons are known to be able to induce skin irritation; several of these compounds were positive in skin irritation testing and are classified as skin irritants.

This section addresses the mechanisms of skin local effects including irritation, corrosion and sensitization and the methods for the determination of these local effects.

5.1. LOCAL SKIN EFFECTS

5.1.1. Skin irritation

Skin irritation encompasses a broad range of sensory and visible effects including dryness, fissuring, erythema, and oedema, which occur as a result of local inflammatory processes following single or repeated contact of the skin with chemicals (Maibach and Coenraads, 1995; Weltfriend *et al*., 1996). Acute irritant contact dermatitis is characterized predominantly by inflammation, while chronic irritant contact dermatitis is characterized predominantly by hyperproliferation and transient hyperkeratosis (Corsini and Galli, 2000). Skin irritation is a complex phenomenon that involves resident epidermal cells, dermal fibroblasts, and endothelial cells as well as invading leukocytes, particularly T-lymphocytes, interacting with each other under the control of a network of cytokines, neuropeptides, and eicosanoids. Keratinocytes presumably play an important role in the pathophysiology via generation of signals leading to attraction of leukocytes (Fuchs *et al*., 2001). The underlying pathophysiological mechanism by which a chemical induces an inflammatory response is not fully understood; however the fact that chemicals of different structure and physico chemical properties can cause skin inflammation implies that different pathways are involved. One of mechanisms by which a chemical can cause skin irritation is damage to a skin barrier for example by extraction of the lipids from the *Stratum corneum* by lipophilic solvents such as aliphatic and aromatic hydrocarbons. Increased water loss due to skin barrier perturbation can initiate release of pro-inflammatory cytokine IL-1α, IL-1 β and tumour necrosis factor (TNF) alpha which induces secondary molecular responses and an inflammatory cascade (Wood *et al*., 1992; Nickoloff and Naidu, 1994; Corsini and Galli, 1998; Welss *et al*., 2004). The damage to the skin barrier might further facilitate the penetration of larger amounts of skin irritants and other chemicals which normally would not be able to penetrate normal intact skin. Chemicals which are able to penetrate the *stratum corne*um may elicit toxicological effect by damaging viable epidermal cells. In response to damage keratinocytes release IL-1α, which essentially is a primary event in skin defence. IL-1α stimulates keratinocytes and fibroblasts to produce and release more IL-1α and other pro-inflammatory cytokines and chemokines such as IL-1β, IL-6, and tumor necrosis factor (TNFα) as the first step in the inflammatory cascade. These cytokines and chemokines in turn induse production of a wide array of other inflammatory meadiators and adhesion molecules resulting in the recruitment and proliferation of leukocytes at the site of inflammation (Nickoloff and Naidu, 1994; Corsini and Galli, 2000; Welss *et al*., 2004; Homey *et al*., 2006).

Furthermore, contact irritants can induce skin inflammation by generation of reactive oxidative species in the skin which might activate transcription factors inducing synthesis of proinflammatory cytokines, dysregulate redox-sensitive signal transduction pathways, and trigger cytotoxicity and apoptosis (Corsini and Galli 1998; Fuchs 2001; Allen and Tresini, 2000; Coleman, 2003; Welss *et al*., 2004).

Acute inflammation is a rapid self-limiting process; however, by repeated exposures it may be maintained for a prolonged time and/or become chronic (Lisby, 2006). In addition to development of chronic contact dermatitis, repeated irritation can lie at the basis of the progression-promoting effect in the development of skin tumours. There is increasing evidence that severe, long-term dermal irritation, necrosis and degeneration induced by petroleum fuels maybe integrally related to possible tumorgenesis (Ingram and Grasso, 1991; Freeman *et al*., 1993; Walborg *et al*., 1998; Nessel *et al*., 1998, 1999a,b; Nessel, 1999). Although mechanisms for this effect are not yet clear, it has been hypothesized that epidermal hyperplasia with repeated episodes of damage and regeneration can initiate tumor induction due to oncogene activation possibly stimulated by release of oxidative enzymes from inflammatory cells.

5.1.2. Skin corrosion

As defined by the United Nations (UN) Globally Harmonised System for the Classification and Labelling of Chemical Substances and Mixtures (GHS) skin corrosion refers to the production of irreversible damage to the skin, manifested as visible necrosis through the epidermis and into the dermis, following the application of a test material.

5.1.3. Skin sensitization

Dermal exposure to contact allergens induces highly regulated cellular and molecular events that result in the stimulation of cutaneous immune responses and the acquisition of skin sensitization. In contact allergic reaction, a distinction should be made between induction (sensitization) and effector (elicitation) phases (Rustemeyer, 2006). The induction phase includes the events following a first contact with the allergen and is complete when the individual is sensitised and capable of giving a local immune response. The effector phase begins upon elicitation (challenge) and results in a clinical manifestation (Rustemeyer, 2006). Most contact allergens are small, chemically reactive molecules with a molecular weight less than 500 Da (Bos and Meinardi, 2000). Upon penetration through the epidermis, a chemical may react with protein or peptide, and then subsequently be processed by epidermal Langerhans cells (LC), a step which is critical for development of sensitization. Allergen-carrying LC become activated and travel to the draining lymph nodes: the site of proliferation for specific T-cells. Migration and maturation of LC following skin sensitization is mediated by release of locally available cytokines and chemokines such as IL-1β, TNF-α and IL-18 (Ryan *et al.*, 2007a). Sensitizing potential is dependent on several factors including the molecule's capacity to penetrate the *Stratum corneum*, lipophilicity and chemical reactivity. Two other factors which further contribute to the allerginicity are pro-inflammatory activity and capacity to induce maturation of Langerhans cells. There are clearly dose-response relationships for both induction and elicitation phases which are dependent on the potency of the chemical allergen but also on external factors such as influence of vehicle or formulation. (Ryan *et al*., 2007 b)

5.2. METHODS FOR THE ASSESSMENT OF LOCAL SKIN EFFECTS

5.2.1. Test methods for skin irritation and skin corrosion

The assessment of the irritancy/corrosion potential of chemicals is important when establishing the hazard classification of industrial chemicals and for safety-assessment purposes. Various *in vivo* and *in vitro* tests which will be addressed in this section are used to determine the irritation and corrosion hazard resulting from the contact of skin with chemicals. Due to complex mechanisms and different pathways of skin irritation, different endpoints are often needed to evaluate the irritation potential of chemicals.

At present, validated *in vivo* and *vitro* tests for irritation and corrosion tests are available, and some of them are accepted by regulatory bodies (OECD TG 404, 1999, 2002; US-EPA 1996). The OECD guideline 404 (OECD, 1992, 2002) covers the assessment both of skin corrosivity (classified as R34 or R35) and skin irritation (classified as R38) based on *in vivo* rabbit skin tests. The criteria for R35 (causes severe burns) indicate that the substance will cause full thickness skin destruction within 3 min; R34 (causes burns) indicates that full thickness skin destruction follows an exposure of up to 4 h. For R38 (irritating to skin), a substance will cause significant inflammation of the skin, which persists for at least 24 h after a semi-occluded exposure of up to 4 h.

The OECD has indicated that *in vitro* toxicity tests can be accepted for regulatory purposes only after a successful experimental validation study (Liebsch and Spielmann, 2002). Two *in vitro* models for skin corrosion have formally been accepted by regulatory bodies; the Transepicutaneous resistance test (TER), based on measuring of the electrical resistance in ex *in vivo* rat skin and the Human Skin Model Test using a reconstituted human epidermal model (TG 431) (OECD, 2004c). In addition to the TER test and human skin models, the Corrositex membrane barrier test has been proposed for assessing the dermal corrosion hazard potential of chemicals (TG 435) (OECD, 2006).

Although several *in vitro* test methods for assessment of skin irritation are under evaluation, at the time of writing this report, none of them have been approved as a full alternative for *in vivo* tests. Some of these methods have been shown to be useful in studying skin irritation of various types of substances for the purpose of internal testing, risk assessment and screening (Faller, 2002). Moreover, some of these tests have passed the last stage of evaluation and have been submitted for formal approval by the regulatory bodies (Cotovio *et al*., 2005; ESAC, 2007; Kandarova *et al*., 2005).

The principles of common *in vivo* and *in vitro* tests will be addressed below.

In vivo methods for skin irritation and corrosion

The *in vivo* rabbit skin test for irritation and corrosion has served as the basis for international regulatory requirements for the testing of chemicals (OECD, 1992, 2002). This test consists of the topical application of substances to the albino rabbit's dorsal skin and the subsequent assessment of induced skin damage. The duration of the study should be sufficient to evaluate the reversibility or irreversibility of the effects observed. A scoring system based on the intensity of erythema and oedema enables products to be classified from non-irritant to very irritant. The dermal irritation scores should be evaluated in conjunction with the nature and severity of lesions, and their reversibility or lack of reversibility.

As rabbit skin is physiologically different from human skin, there has been criticism that the test cannot accurately predict irritation potential in humans (Robinson *et al*., 2000; Robinson and Perkins, 2002).

For chemicals that lack other toxicities (e.g. mutagenicity, sensitization, corrosivity etc.) 4-h patch test with human volunteers has been developed (Basketter *et al*., 2004; Robinson *et al*., 2001). To avoid subjective scoring of swelling or redness, alternative parameters have been introduced such as cutaneous blood flow, as measured by Laser Doppler Flowmetry, infrared detection of skin temperature, and skin thickness assessment. In addition to methodology used for hazard identification, skin irritation has been studied in experimental studies in volunteers. Besides measurements of visible skin effects such as erythema and oedema, the profile of cytokines and other inflammatory mediators have been measured in human volunteers by using punch biopsies, suction blister techniques, and *Stratum corneum* tape stripping techniques (Ulfgren *et al*., 2000; Perkins *et al*., 2001; de Jongh *et al*., 2006).

***In vitro* prediction of skin irritation and corrosion**

The development of *in vitro* alternatives for skin irritation and corrosion has been driven by scientific and ethical considerations (Botham *et al*., 1998). Due to a limited supply of fresh human skin, a variety of *in vitro* systems have been developed, including animal ex-*in vivo* models and keratinocyte cultures. The Skin Irritation Function Test (SIFT) (Heylings *et al*., 2003) and the Pig Ear Test (PET) (Fentem *et al*., 2001) are two examples of *ex vivo* animal models (mouse and pig, respectively). Keratinocytes can be cultured as a monolayer or as stratifying layers, that can also be grown in conditions where the cultured cells reconstruct the basis of an epidermis with three dimensional organization and production of a cornified barrier when the surface of the culture is exposed to the air. Keratinocytes monolayers have been widely used to study dermal irritancy. However, due to the absence of the principle barrier, the *Stratum corneum*, use of this model leads to higher exposure of keratinocytes (Ponec and Kempenaar, 1995, Coquette *et al*., 2003).

Currently, the most relevant *in vitro* tests for regulatory purposes are based on reconstituted human skin equivalents. The reconstituted human epidermis is a multilayered human skin grown in the laboratory which mimics the biophysical properties of *in vivo* human epidermis. The reconstructed epidermis model presents the following mechanistic advantages: (1) it mimics the architecture of a normal epidermis, where the presence of the *stratum corneum* is obtained through an imersion step (required for keratinocyte differentiation); (2) it can be used to assess biological parameters underlying various types of damage; and (3) it permits the topical application of chemicals or finished products directly onto the *stratum corneum* (Borlon *et al*., 2007). Different end-points can be assessed including tissue viability, histological evaluation, the release of inflammatory mediators and expression of genes taking part in stress response such as cell signalling, inflammation, protein metabolism, etc.

In vitro skin corrosion and *in vitro* skin irritation assays differ significantly in their test design concerning exposure pattern, follow up time and investigated end point. In comparison to *in vitro* skin corrosion test, the design of *in vitro* skin irritation protocols enables long-term development of the cell damage or tissue recovery after exposure to a test chemical. Corrosive materials are identified by their ability to produce a decrease in cell viability by using an appropriate test such as MTT-assay (Mosman, 1983). The cut-off percentage cell viability value distinguises corrosive from non-corrosive test materials.

Unlike corrosion, skin irritation encompasses a vast range of severity. In contrast to *in vivo* irritation tests which primarily rely on visible changes in the skin such as erythema and erythema, irritation *in vitro* tests assess various biomarkers for different end-points such as cytotoxicity, morphological changes, differential expression of genes, release of inflammatory cytokines (IL-1 and TNF-α) and neuropeptides (substance P (SP)) (Coquette *et al.*, 2003; Tornier *et al*., 2006; Chateerjee *et al*., 2005). Cytotoxicity is known to trigger irritation processes, and can therefore be a first common event for irritants. Cell cytotoxicity assays such as cell viability (e.g. MTT conversion and the membrane integrity – such as neutral red-uptake or LDH release) are amongst the most common *in vitro* endpoints used to predict the toxicity of a substance in cell culture. Although there is a correlation between irritation potential and cell viability, measurment of cytotoxicity alone does not always discriminate between irritants and non-irritants (Fentem *et al*., 2001) and it has been recommended that more specific biomarkers, that have different mechanistic response, should be incorporated. For this purpose, the release of a membrane damage marker, adenylate kinase (AK), and of cytokines IL-1α and IL-8 were also investigated. Combining these endpoints, a simple two-tiered strategy was developed, with the MTT assay as the first, screening stage. This resulted in a clear increase in sensitivity, and a fall in the false-positive rate (Cotovio *et al*., 2005).

Today several reconstructed human skin models are commercially available, of which the EPISKINTM model (EPISKIN-SNC, France), the EpiDermTM model (MatTek Corporation, MA, USA) the SkinEthicTM model (SkinEthic Laboratories, France), EST-1000TM (Cell Systems GmbH, Germany) and Phenion TM (Phenion GmbH, Germany) are the most used.

For skin corrosion, in addition to Human Epidermal Model, two other tests are available which have been formally accepted as *in vitro* alternatives for *in vivo* skin tests. The Transcutaneous Electrical Resistance (TER) Assay (OECD, 2004) is based on an irreversible loss of normal *stratum corneum* integrity and function measured as a reduction in the inherent TER below a predetermined threshold level. A test material is topically applied for up to 24 hours to the epidermal surfaces of skin discs obtained from humanely killed young rats. A validation study and other published studies have reported that the TER assay is able to reliably discriminate between known skin corrosives and non-corrosives, and there was good agreement between the skin corrosivity classifications derived from the *in vitro* and *in vivo* data assessed under OECD guideline 404 (OECD, 2002). However, the TER test does not allow the sub-categorisation of corrosive substances. Another *in vitro* corrosivity test is CORROSITEX in which the potential corrosivity of a test material is assessed by measuring the time required for a chemical to ‘breakthrough’ a bio-barrier membrane (a reconstituted collagen matrix). The average breakthrough time of four replicates is used to determine whether a chemical is corrosive or not.

5.2.2. Test methods for skin sensitization

Under current regulations, the identification of skin sensitization hazard is assessed through *in vivo* testing. The animal test methods used in harmonised classification of substances, according to their potential to cause skin sensitisation, are the guinea pig maximization test (GPMT), the Buehler test, and the local lymph node assay (LLNA) (ref OECD 406, OECD 429, EU B.6, EU B.42, UNECE GHS). A substance that is classified as a skin sensitizer carries the Risk Phrase R43 according to EC criteria. A substance is either a skin sensitiser or not and there is no subdivision based on potency.

Substances are classified as skin sensitizers if, in properly conducted tests, at least 30 % of animals show a positive response in a GPMT and 15 % in a Buehler test (Buehler, 1965). For the LLNA a positive response is defined if at least one concentration induces a 3–fold or greater increase in proliferation in draining lymph nodes compared with concurrent vehicle-treated controls (the EC3 value).

The guinea pig maximisation test (Magnusson and Klingman, 1970) and the occluded patch test (Buehler, 1965) are the most widely applied animal models. In these tests, groups of animals (guinea pigs) are topically exposed to the test material. In some tests, an adjuvant is also administered to enhance immune responses. Elicitation of cutaneous hypersensitivity is determined as a function of challenge-induced erythema and/or edema. Sensitizing potential is judged on the basis of the frequency of specific reactions induced by this challenge. One of the drawbacks of the guinea pig tests is that it doesn't provide information on relative potency (ECETOC 2000). In this regard, important progress has been made by development of the LLNA (Kimber and Basketter, 1992; Gerberick *et al*., 2007; Basketter *et al*., 2007). This test is based on the events induced during the induction phase of skin sensitization. Skin sensitizers are identified as a function of their ability to provoke lymphocyte proliferation in the draining lymph nodes following topical exposure of mice to chemicals. This method, which has fully been validated as an alternative to guinea-pig tests, can be used not only for hazard identification but also for assessment of the relative skin sensitizing potency of a substance (ECETOC, 2003; Basketter *et al*., 2007; Gerberick *et al*., 2007).

5.2.3. Models for the prediction of skin sensitization

In vitro and *in silico* models provide an alternative approach for the assessment of skin sensitization potential but as yet there are no validated methods which are acceptable for regulatory purposes. For the development of *in vitro* tests, changes in expression of cell surface markers (e.g., CD54, CD80, CD83, CD86) and chemokine receptors (e.g. CXCR4, CCR5 and CCR7) have been investigated by using peripheral blood-derived dendritic cells (Rustemeyer *et al*., 2003; Aeby *et al*., 2004; Staquet *et al*., 2004; Boisleve *et al*., 2004). However, to establish this as a validated *in vitro* assay for skin sensitization, further optimization and development are needed.

Another potential alternative approach to skin sensitisation hazard identification is the use of QSARs. QSARs for skin sensitisation comprise so-called 'local' models and 'global' (or general) models (Patlewicz *et al*., 2007). Local models are typically characterised by a chemical class or by a single chemical mechanism of action. Global models are based on skin sensitisation datasets comprising diverse chemical structures corresponding with a number of mechanisms of action. Expert predictive systems are built upon experimental toxicity results with prediction rules derived from the data. The rules may be based on statistical inference and take the form of QSARs (e.g. TOPKAT, MultiCASE), or based on expert judgment taking the form of SARs describing reactive chemistry (e.g. Derek for Windows (DfW)) or they may be a hybrid of the two approaches (e.g. TIssue MEtabolism Simulator (TIMES)). At present, none of the existing approaches seemed to be suitable for routine prediction of skin sensitisation hazards of chemicals. Further work is needed before validated QSARs for this endpoint are available (Li *et al*., 2007).

5.3. SUMMARY OF THE METHODS FOR THE ASSESSMENT OF LOCAL EFFECTS OF PETROLEUM HYDROCARBONS

In vivo methods to assess skin irritation include measurements of skin barrier (e.g. TEWL), skin inflammation (erythema and oedema), and release of inflammatory mediators. At present, with the exception of corrosion, no alternative *in vitro* tests for skin local toxicity are available for regulatory purposes, although some of reconstructed human skin models have passed the last evaluation stage and have been submitted for formal approval by regulatory bodies. Although these models are one of the most promising *in vitro* systems that have the potential to replace animal assays for assessment of skin corrosion and irritation it must be mentioned that skin equivalents differ from normal human skin in some characteristics. Most models consist of keratinocytes and the other skin cells which play a critical role in inflammatory response such as Langerhans cells are absent. Moreover, the absence of blood-derived and resident leukocytes reduces the complexity of the observable cytokine network (Coquette *et al*., 2003). Furthermore, skin equivalents showed approximately 10–30-fold higher permeability as compared to normal skin which might lead to an over-prediction of irritants due to the higher penetration rate of applied substances (Perkins *et al*., 1999). It seems also that the reconstructed epidermal models are more sensitive to some families of compounds (Tornier *et al*., 2006). To date, alternative approaches to the *in vivo* skin tests have proved largely successful at identifying severe irritants, but are generally poor at discriminating between agents with mild to moderate irritant potential. Identification of new more specific markers of irritation will improve the applicability of *in vitro* irritation test. For skin sensitization the LLNA has been shown to be a valuable alternative to *in vivo* animal models for hazard identification and also for assessment of relative skin sensitizing potency of a substance.

For asessment of local toxicity of petroleum hydrocarbons various *in vivo* and *in vitro* methodologies have been used. For the regulatory purpose, usually *in vivo* rabbit skin tests have been conducted. For research purposes, *in vitro* systems based on reconstructed epidermis are increasingly being used, but using different toxicological endpoints. One of the concerns about using these systems is their limited barrier function. Since various studies have shown the importance of local tissue concentration, this should be considered in the design of the experiment and interpretation of the results. Another significant problem with tissue culture systems is the necessity for solubilizing the lipophilic hydrocarbons into aqueous media which can be attained by addition of various solubilizers. Furthermore, some of petroleum hydrocarbons are volatile and can evaporate rapidly from the exposure medium affecting the local concentration at the site of action. It is clear that all these experimental factors might influence the outcomes of the study.

6. LOCAL SKIN EFFECTS OF PETROLEUM HYDROCARBONS

The primary irritation hazard of a number of petroleum hydrocarbon has been assessed. As seen from **Table** III, a number of petroleum hydrocarbons were classified as skin irritants (R38) as of the time of writing this report, although in some cases this is based on structure-activity considerations rather than actual laboratory data (**Table** III).

Table III Results of Dermal Irritation (Draize Skin Irritation Tests) of Key Gasoline Constituents

Substance	Summarised Result
n-Hexane	Classified Xi; R38
Cyclohexane	Not irritating to rabbit skin when applied under non-occlusive patches, but more severe and persistent scores when a chamber was utilised to prevent evaporation. (classified Xi;R38)
Benzene	Grade two erythema which progressed to grade 3
Heptane	No data (classified Xi;R38)
Methyl Cyclohexane	Produces local irritation and thickening with repeated dermal application
Toluene	Grade 2 erythema observed which did not clear within 7 days.
Octane	Classified Xi;R38 No data
Ethyl Benzene	Considered "moderately irritating to the skin"
Xylenes	Classified Xi;R38
Tri-methyl Benzene	Classified Xi; R38
n-Propyl Benzene	Considered "slightly irritating" (isopropyl benzene as a surrogate)
Naphthalene	Naphthalene is considered to be a "slight" skin irritant and is not classified.

The mechanism of the irritation has been studied in more detail for several individual hydrocarbons and petroleum products. The most investigated individual hydrocarbons are aromatic solvents toluene and xylene. Prolonged skin contact with these aromatic hydrocarbons can lead to burning sensation, erythema, delipidization, and dermatitis (see for review Rowse *et al*., 2004). Occluded exposure to toluene, for as little as 10 min, has been documented to significantly increase the blood flow *in vivo* in humans (Mahmoud and Lachapelle, 1985). This is in agreement with human volunteer study of Kezic *et al*. in which skin irritation was observed after short-time (3 min) exposure to neat toluene resulting in enhanced absorption rates (Kezic *et al*., 2001). In another study in human volunteers, dermal application of toluene caused erythema, increase in TEWL and skin blood flow and a decrease in skin moisture content (Frosch and Kurte, 1994). Toluene is often used in experimental irritation studies as a model skin irritant. The degree of induced irritancy is preparation-, dose- and exposure-time dependent with symptoms ranging from very faint erythema to severe irritation in humans (Frosch and Kurte, 1994).

Also animal studies show that these aromatic solvents may act as skin irritants. Histological changes in guinea pig skin due to exposure to toluene after a 2-h occlusive application showed degenerated epidermal cells such as pyknosis of nuclei and perinuclear oedema. (Mahmoud *et al*., 1984)

In a recent study (Ahaghotu *et al*., 2005) using hairless rats *in vivo* the effect of methyl substitution of benzene on the skin irritation was assessed by measuring TEWL, erythema, skin histopathology and expression of interleukin-1α (IL-1 α) and tumor necrosis factor- α (TNF-α) in the skin and blood. Rats were exposed dorsally to neat chemicals (dose of 15 µl every 2 hours for 8 hours a day for four days over an area of 3 cm^2). Test chemicals used were benzene, xylene and tetramethylbenzene (TMB). As measured by TEWL all chemicals significantly disrupted skin barrier compared to controls (at 24 h TEWL values for controls, benzene and xylene, and TMB were 5.59 ± 0.52, 11.3 ± 1.66, 11.14 ± 0.90 and 8.15 ± 1.00 gm^{-2} hr, respectively). Although TMB induced relatively lower TEWL than xylene and benzene at 24 h, it exceeded benzene and xylene at 32 h and remained at a higher level throughout 104 h (16.82 ± 2.42 g m^{-2} hr). Unocclusive exposure resulted also in mild skin irritation; xylene induced slightly higher irritation then benzene and TMB (0.8 ± 0.20, 0.4 ± 0.24 and 0.40 ± 0.16, respectively). As irritation increased with time during the application, TMB demonstrated highest irritation score of 2.6 ± 0.16 at 104 h. Both TEWL and erythema demonstrated increases with the lipophilicity or methyl substitution of the aromatic hydrocarbons. Skin histopathology revealed only slight swelling and disruption of the *stratum corneum* due to exposure to benzene, while besides swelling and disruption, exposure to xylene induced granulocyte infiltration in the epidermis and infiltration of homogenous eosinophilic material at epidermal-dermal separation which is in agreement with the findings of Gunasekar *et al*. (Gunasekar *et al*., 2003). Most severe damage was induced by TMB. Additionally, TMB induced high increase of mast cells in the areas of inflammation which is thought to be an immunological reaction due to exposure to TMB. High levels of IL-1α were only found in the blood suggesting rapid clearance of the cytokines from the skin. Almost 12 fold levels of IL-1α by TMB indicate severe irritation response of the skin. In contrast, TNF- α was only found in the skin indicating its local accumulation induced by irritation and inflammation (being highest for TMB; 11-fold in comparison to benzene and xylene; 5- and 6-fold, respectively).

The effects of short-term and long-term occlusive and unocclusive dermal exposure to xylene and benzene on the skin irritation response, skin moisture content, erythema and cytokine/chemokine levels were investigated in hairless rats *in vivo* (Chatterjee *et al*., 2005). The dorsal skin of rats was exposed occlusively (short-term exposure) to 230 µl of xylene or benzene over an area of 1.04 cm^2 for 1 hour. Unocclusive (long-term exposure) repeated exposure was performed by application of 15 µl of xylene or benzene every 2 h for 8 h a day, for four days over an area of 3 cm^2. The measurements were performed at different time points before, during (only unocclusive exposure) and after the end of exposure. For the assessment of biomarkers in skin and blood, blood samples were collected at 24 and 104 h. As measured by TEWL both chemicals significantly disrupted the skin barrier upon occlusive and unocclusive exposure (TEWLs at 1 h under occlusive conditions were 11.3 ± 1.66 and 15.96 ± 2.21 g m^{-2} hr and at 24 h under unocclusive conditions were 11.3 ± 1.66 and 11.14 ± 0.90 g m^{-2} hr for benzene and xylene, respectively; control TEWLs were 6.20 ± 0.45 and 5.59 ± 0.52 g m^{-2} hr for occlusive and unocclusive exposure, respectively). Skin moisture content due to occlusive benzene exposure was lowered after 7 h being significantly lower than control at 8 h and 24 h, while xylene induced consistently lower skin moisture content. Prolonged unocclusive exposure to benzene and xylene induced a low level of moisture content throughout

the experiment (104 h). The effect of the short-term occlusive exposure to benzene and xylene showed that xylene is more irritating than benzene to hairless rat skin (at 24 h score was 1.8 ± 0.2 and 1.2 ± 0.2 for xylene and benzene, respectively). Unocclusive long term exposure to benzene and xylene induced mild irritation within 8 h which steadily increased with time and reached maximum score of 1.8 ± 0.2 and 2.0 ± 0.32 for benzene and xylene, respectively. Occlusive exposure induced 2.4- and 2.7-fold levels of IL-1α, while unocclusive exposure induced 3.7 and 3.9-fold increased levels of IL-1α for benzene and xylene, respectively. Exposure to benzene and xylene induced similar increase of TNF- α in the skin: 2.3- and 2.5-fold for occlusive exposure and 6.2- and 5.7-fold for unocclusive exposure. Both occlusive and unocclusive exposure elevated monocyte chemoattractant protein-1(MCP-1) levels in the skin approximately by 1.7 fold compared to the control. Presented data showed that repeated long-term, low-level exposure is more harmful than short-term, high-level exposure in respect to benzene and xylene. Furthermore, there was a significant difference in irritation potential between occlusive and unocclusive exposure.

Gunasekar *et al*. (2003) studied histological and molecular changes in the skin that may reflect induced dermal irritation as a response to exposure to neat m-xylene. Hairless rats were exposed to m-xylene on their backs over an area of 4.9 cm^2. The skin sites were excised at zero, one, two, four and six hours after the beginning of the exposure. As molecular marker of inflammation interleukin-1α (IL-1α) and inducible nitric oxide synthase (iNOS) proteins were measured. Local skin damage due to exposure to m-xylene was suggested by accumulation of homogenous eosinophilic material at the areas of epidermal-dermal separations. Exposure to m-xylene also induced increase of IL-1α (50-85% above the control samples, maximum at 1-2 hours) and iNOS protein (15-60% above control skin samples with a maximum at 4 hours).

Rogers *et al*. (2001) studied hairless rats (6-10 per group) *in vivo* whether oxidative species and low molecular weight (LMV) DNA could be detected in the skin following dermal exposure to m-xylene. Hairless rats were exposed to m-xylene over an area of 4.9 cm^2 for 1 hour. The skin sites were excised at zero, one, two, four and six hours after the beginning of the exposure. Oxidative species, determined by measuring the oxidation of 2',7'-dichlorofluorescin diacetate, were found in both exposed and unexposed skin being higher in exposed sites (1.4 - 2.0 fold greater than unexposed site). There were also high levels of low molecular weight DNA present in both exposed and unexposed skin being significantly higher in the skin excised after two, four and six hours (110.9 – 588.5 ng LMV DNA/µg genomic DNA) .

In another study (Singh *et al*., 2003) the local skin effect of dermal exposure to xylene, heptane and hexadecane was investigated *in vivo* in weanling pigs. Weanling pigs were exposed to neat chemicals for 24 h over an area of 1 cm^2. Measurements of TEWL and erythema were performed at zero h, 1 h, 2 h and 24 h after the end of exposure. Heptane caused significant increase in TEWL (2-fold compared to control) while hexadecane and xylene did not produce any significant changes in TEWL at any time points after the end of exposure. Slight erythema was observed at all chemically treated sites and it was completely resolved at sites treated with hexadecane and xylene, while elevated erythema persisted at sites treated with heptane 24 h after the end of exposure. None of the investigated hydrocarbons caused edema.

The effect of unocclusive dermal exposure to nonane, dodecane and tetradecane on skin irritation was investigated in hairless rats *in vivo* by measurement of

erythema and TEWL. Furthermore, expression of IL-1α, TNF-α and monocyte chemoattractant protein-1 (MCP-1) were determined in the skin and blood at the end of dermal exposure (Babu *et al.*, 2004). The chemicals were applied unocclusively every 2 h for 8 h a day for four days over an area of 3 cm^2. All the chemicals demonstrated a significant increase in TEWL during the exposure period; the increase in the TEWL was in the following descending order: tetradecane> dodecane> nonane. Also the erythema scores increased with increase in the molecular weight. Nonane and dodecane caused moderate erythema while tetradecane produced severe erythema. The expression of IL-1α in the blood and TNF-α in the skin was higher for tetradecane followed by dodecane and nonane. These results are in contrast with those reported by the same research group (Babu *et al*., 2004a) in which single occlusive application of these chemicals produced higher irritation by nonane than dodecane and tetradecane as assessed by TEWL and erythema. Tetradecane did not show any visible signs of skin irritation and also did not produce any significant difference in IL-1α and MCP-1 as compared with the control. Nonane significantly increased the expression of IL-1α, TNF-α and monocyte chemoattractant protein-1 (MCP-1) in skin and blood as compared to the control. Tetradecane and dodecane did not elicit IL-1α release either in the skin or in blood, whereas nonane exposure showed higher IL-1α levels in blood. The expression of TNF-α was higher in response to all the chemicals but significantly more with nonane than dodecane or tetradecane.

Expression of a number of genes involved in inflammatory and growth-related pathways has been studied in rats *in vivo* after brief dermal exposure to JP-8 and 4 of its constituents: undecane (UND), tetradecane (TET), trimethylbenzene (TMB) and dimethylnaphtalene (DMN) by using a microarray technique (McDougal and Garrett, 2007). Results of this study revealed that all investigated chemicals caused responses that may result in irritation. UND and TMB showed the greatest number of gene changes, more than twice as many as DMN, and about 10-fold more than TET. UND caused changes in development, morphogenesis, and cell differentiation whereas TMB caused dramatic transcript decreases in metabolism categories. Differences in gene expression between investigated compounds showed not to be caused by different epidermis concentrations.

Release of neuropeptide P (SP) as a proinflammatory biomarker was measured after topical occlusive exposure to xylene in rats *in vivo* by using a microdialysis technique (Fulzele *et al*., 2007). Occlusive exposure to xylene produced significant SP release after 2 and 3 h following removal of occlusion, however no significant induction of SP was observed during the period of occlusive treatment.

An *in vivo* study was conducted with Yucan minipigs to evaluate dermal toxicity of neat JP-8, nonane or toluene (Kanikkannan *et al*. 2001a). TEWL, skin capacitance (moisture content), erythema and edema were evaluated before and after treatment. Application of toluene increased the TEWL by 1.4 times at 2 h after the removal of patches and the TEWL value remained high until 24 h though it was statistically insignificant compared to base line level ($p > 0.05$). Exposure of pig skin to nonane caused a gradual increase in the TEWL during the 24 h post application period and the TEWL was about 2 times higher at 24 h than the baseline value ($p < 0.05$). Both toluene and nonane caused slight erythema. Application of toluene did not cause a significant edema whereas nonane caused a slight edema, which gradually decreased after 24 h following removal of the patches.

Muhammad *et al*. (2005b) assessed the irritation caused by JP-8 and eight aliphatic hydrocarbons (nonane, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane) and 6 aromatic hydrocarbons (ethyl benzene, o-xylene,

trimethyl benzene, cyclohexyl benzene, naphthalene, dimethyl naphthalene) which were topically exposed to pigs for 1 day and with repeated daily exposures for 4 days. Erythema, epidermal thickness, and epidermal cell layers were determined. Erythema and epidermal hyperplasia were pronounced after 4 days of repeated application of the aliphatic hydrocarbons. Aromatic hydrocarbons such as ethyl benzene, o-xylene, trimethyl benzene, cyclohexyl benzene, naphthalene, and dimethyl naphthalene did not produce any macroscopic or significant microscopic changes in epidermal thickness or lesions after 1 or 4 days of *in vivo* exposures. Morphological observations revealed slight intercellular and intracellular epidermal edema in 4-day exposures with the aliphatic hydrocarbons. Ultrastructural studies showed that jet fuel hydrocarbons-induced cleft formation within intercellular lipid lamellar bilayers of the *stratum corneum*. The degree of damage to the skin was proportional to the length of *in vivo* hydrocarbons exposures. A hydrocarbon-specific response was demonstrated by epidermal thickness and the number of epidermal cell layers, with tridecane and tetradecane having the greatest proliferative effect followed by JP-8 and pentadecane after 4 days of *in vivo* exposures. The short chain aliphatic hydrocarbons such as nonane, decane, and undecane produced only mild erythema after 4 days of *in vivo* exposures. These findings are in accordance with Brown and Box (1970) who studied the skin irritancy of alkanes and reported that *n*-decane was slightly irritant with some epidermal thickening, while n-tetradecane was more irritant with epidermal thickening. The same research group (Muhammad *et al*., 2005b) reported that individual hydrocarbons may cause lipid extraction from the *stratum corneum*, as studied with Fourier Transform Infra Red (FTIR) spectroscopy and transmission electron microscopy (TEM) (Monteiro-Riviere *et al*., 2004).

In several *in vitro* studies, various biomarkers of skin irritation were investigated after exposure to aliphatic and aromatic hydrocarbons providing more insight in the molecular mechanisms of the inflammatory effects.

Oxidative damage from dermal exposure to the vapours of xylene and toluene was investigated in human skin *in vitro* (Costa *et al*., 2006). Skin integrity was assessed by measuring TEWL and percutaneous penetration of a model compound 1,2,4-trimethylbenzene. In addition to skin viability as assessed by the MTT test, the activity antioxidant systems, including glutathione transferase, catalase and superoxide dismutase, were determined. As biomarkers of oxidative stress, products of lipid and protein peroxidation were measured in the skin. 8-h dermal exposure to vaporous xylene and toluene significantly reduced tissue viability when concentrations higher then 10^4 ppm. The overall trend of the results indicates that both solvents can damage human skin even at concentrations comparable to their TLV, and that oxidative stress has a role in these effects.

The toxicity of the JP-8 components m-xylene, 1-methylnaphthalene (1-MN), and n-nonane was studied in the murine keratinocyte line (Rogers *et al*., 2004). The viability of keratinocytes was assessed by measuring reduction of MTT and was expressed as the EC_{50} value (chemical concentration at which 50% cell viability was observed). The viability of keratinocytes exposed to m-xylene, 1-MN, and n-nonane decreased with increasing chemical concentration. The results show that m-xylene is the most potent, followed by n-nonane, and 1-MN as the least potent, supporting earlier findings that the aromatic hydrocarbons cause greater direct cytotoxicity than aliphatic hydrocarbons (Chou *et al*., 2003).

The cytotoxic effect of m-xylene in rat dermal fibroblast and the role of oxidative stress were studied by Coleman *et al*. (2003). Viability of fibroblast in dermal equivalents was assessed using MTT assay. Levels of two cellular antioxidants,

endogenous catalase and endogenous thiols, were assessed spectrophotometrically and by using Ellman's reagent, respectively. The results show that m-xylene induces cytotoxicity as assessed by decreased viability of the fibroblast with calculated EC_{50} value to be 1481.1 ± 88.2 and 930 ± 32.5 µg m-xylene/g cell at 1 and 4 hour exposure, respectively. The observed EC_{50} at 1 hour exposure was about 60% higher than the EC_{50} at 4 hour exposure. M-xylene was also shown to promote decreases in cellular antioxidant levels in time- and dose-dependent manner. The maximum observed decrease in thiol level was about 20 and 40% (at 1 and 4 hour exposure, respectively) and in catalase activity was about 10 and 55% (at 1 and 4 hour exposure, respectively).

Chou *et al*. (2002) investigated the effect of chain length of individual aliphatic hydrocarbons on cell toxicity and release of IL-8 in human epidermal keratinocytes (HEK) *in vitro*. 10 aliphatic hydrocarbons with carbon (C) length ranging from 6 to 16 were applied neat on HEK for 1, 5 and 15 mins. All treatments caused significant dose dependent increase in cell mortality which corresponded with the decrease in carbon chain length. However, the increase in IL-8 release showed a peak in response around C9-C13. Higher cytotoxicity by shorter-chain aliphatic hydrocarbons did not correlate with IL-8 release revealing different mechanisms by which these compounds exert toxicity in cell cultures.

In another study from the same research group (Chou *et al*., 2003) *in vitro* cutaneous toxicity of 9 individual aromatic hydrocarbons was assessed in HEK cells. The cytotoxicity was evaluated by determining the dose causing 50% mortality (LD_{50}) and by the highest non-cytotoxic level (HNTL) (5% of HEK cells mortality). In addition, IL-8 release at selected dose was measured. The increase in cytotoxicity was correlated to the number and the size of the side-chains attached to the aromatic ring. LD_{50} rank order potency was cyclohexylbenzene > trimethylbenzene > xylene > dimethylnaphthalene > ethylbenzene > toluene> benzene. At the LD_{50} dose level ethyl benzene, cyclohexyl benzene (which induced significantly the highest IL-8 level compared to other aromatic hydrocarbons), dimethyl naphthalene and methyl naphthalene significantly induced IL-8 release while other aromatic hydrocarbons induced equivalent or slightly higher IL-8 levels compared to controls. The study showed that ability to cause HEK cell death does not entirely correspond to ability to induce IL-8 release which suggests that different mechanisms of action are responsible for skin toxicity and irritation.

Moloney and Teal (1988) investigated a structure activity relationship for neat n-alkanes in a mouse ear edema model to study the mechanism of cumulative irritancy. Animals were repeatedly applied with 5 µl of n-alkanes in hexane to the whole of the pinna of the ear for zero, 8, 24, 32, 48, 56, 72 and 80 h over four days. Ear edema was quantified by measuring of the thickness of the tip of the pinna at zero, 24, 48, 72 and 96 h after the beginning of the exposure. Control exposure was conducted with pure hexane and resulted in no edema. Dodecane had no effect on the pinna thickness, while tridecane induced significant increase at 96 h. Tetradecane induced the greatest response, while hexadecane, octadecane and eicosane produced progressively diminished responses. Octadecane was the only *n*-alkane which induced a significant response already at 24 h. At 96 h the treated sites were exposed to hydrocortisone which revealed a positive correlation between ear thickness and induced permeability of the ear to hydrocortisone. In an additional cross-over experiment mouse pinna was initially treated with tetradecane and further treated with tetradecane, or dodecane or hexane. Continued treatment with tetradecane again resulted in substantial increased of ear thickness and with dodecane in only modest increase of the ear thickness while hexane treatment resulted in no increase of the thickness of the ear.

Allen *et al*. (2001) studied the effect of individual aliphatic hydrocarbons (undecane, dodecane, tridecane and hexadecane) on IL-8 production by normal human epidermal keratinocytes (NHEK) *in vitro* after exposure for 24 h. The results revealed that *in vitro*, individual aliphatic hydrocarbons are capable of inducing IL-8 release from keratinocytes at concentrations that were determined to be subtoxic to cellular membranes. There appear to be differences among each hydrocarbon with respect to their effects on IL-8 release. At subtoxic doses IL-8 release was increased in decreasing intensity as follows: tridecane (C13) > hexadecane (C16) > dodecane (C12) > undecane (C11) > control. However, these results should be interpreted with caution because these differences might be attributed not only to different effects of aliphatic hydrocarbons but also to variations in complexation or differences in their release from the introducing complex with α-cyclodextrins influencing the actual concentration.

Iyadomi *et al*. (2000) investigated the irritancy properties of several aliphatic and aromatic solvents including toluene, *m*-xylene, and *n*-hexane using a mouse ear thickness model. All investigated solvents produced an increase in ear thickness; the strongest effect was observed for toluene and *m*-xylene. Toluene showed a clear dose-response relationship, however at concentrations under 30 % toluene did not produce signs of skin irritancy. The same author reported plasma extravasation and inflammation in the abdominal skin of hairless rats induced by topical exposure to toluene, *m*-xylene and toluene (Iyadomi *et al*., 1998).

Yang *et al*. (2006) studied cytotoxicity and proinflammatory activity of 10 aliphatic (C6, C8-C16) and 7 aromatic (benzene, ethylbenzene, trimethylbenzene, toluene, xylene, cyclohexylbenzene and dimethylnaphthalene) hydrocarbons in HEK. The study was designed to investigate any interactions among alophatic and aromatic hydrocarbons in order to understand how these compounds cross-react in a mixture situation similar to the actual composition of jet fuels. Two parameters representing direct cytotoxicity to the living cells (HEK mortality) and the activation of proinflammatory pathways (IL-8) were evaluated. The application solution was dermally applied as a mixture of aliphatic or aromatic solvents or as a mixture of JP-8 fuel and a single hydrocarbon. The results suggested that middle-ranged aliphatic components (C10–C13) are likely to be the principal hydrocarbons dictating HEK toxicity, while C14–C16 may be the most important components in composing a less dermatotoxic fuel. Increase of aliphatic components in JP-8 generally reduced HEK mortality in a dose-dependent manner. Interestingly, when aliphatic and aromatic hydrocarbons were dosed in combination, the mixture may exhibit a different cytotoxicity or the degree of toxicity may change significantly. Whether or not the changes follow certain patterns or changes in a predictable way is not clear. The results suggested that the significance of aromatic components in a complex mixture like JP-8 seemed to be well below that of the aliphatic components in terms of dermatotoxicity.

Dermal sensitization studies of petroleum hydrocarbons

Petroleum hydrocarbons which have been tested for allergic contact dermatitis either in laboratory animals or in human volunteers do not appear to be contact sensitizing agents (CONCAWE 2009).

6.1. SUMMARY OF THE LOCAL SKIN EFFECTS OF PETROLEUM HYDROCARBONS

There are several lines of evidence that show dermal exposure to both aliphatic and aromatic carbons leads to local skin irritation. Development of a local skin effect is dependent on the local concentration of a chemical in the skin and its intrinsic potential to cause effects on skin structure and induce an inflammatory response. While aromatic hydrocarbons penetrate the skin better than aliphatics, the aliphatic components are absorbed into and remain in the skin to a greater extent than the aromatics which can cause their gradual accumulation in the skin in repeated exposure. In general, aromatic compounds are more irritating to the skin than aliphatic compounds (Boman 1996; Hoekstra and Phillips 1963; Klauder and Brill 1947, Yang *et al*., 2006). The aromatic hydrocarbons are more potent in causing keratinocyte cell death; methyl substitutions make benzene rings more cytotoxic (Ahaghotu *et al*. 2005) and reactivity increases with the number of the side chains attached to the aromatic ring. On the other hand, the aliphatic hydrocarbons are more potent at inducing the release of proinflammatory cytokines. In general, straight chain hydrocarbons are more irritating than branched hydrocarbons with the same number of carbon atoms. The skin irritating potential of aliphatic hydrocarbons increased with increase in the molecular weight and paralled their affinity to the *stratum corneum*. The ranking order of irritant potential of aliphatic hydrocarbons reported in various studies seemed to be dependent on experimental conditions such as occlusion which might influence partitioning and local bioavailability of a chemical. Although several studies showed that for a series of hydrocarbons (C6-C16) irritancy peaked around tetradecane (C14) (Brooks and Baumann 1956; Brown and Box 1970; Moloney and Teal, 1988), a recent study identified such middle ranged aliphatics as the most cytotoxic (Yang *et al*., 2006).

There is increasing evidence that severe, dermal irritation induced by long-term or repeated exposure to certain hydrocarbon fuels can be the basis of the progression-promoting effect in the development of skin tumours. The tumour-promoting activity of alkanes is related to their chain length, with maximal activity found in C12-C14 alkanes.

In contrast to skin irritating effects, there is no evidence that petroleum hydrocarbons cause skin sensitization.

7. CONCLUSIONS: HEALTH RISK OF DERMAL EXPOSURE TO PETROLEUM HYDROCARBONS

7.1. SYSTEMIC UPTAKE AND EFFECTS

Dermal uptake of hydrocarbons can occur after skin contact with a petroleum product in either liquid or vapour form. Studies on the dermal absorption of individual hydrocarbons in petroleum products show a clear decrease of dermal absorption with increasing lipophilicity and molecular weight. Accordingly, higher absorption of aromatics (e.g. toluene, xylene, trimethylbenzene, naphthalene) than aliphatics (decane, dodecane, tridecane and hexadecane) has been reported. Further, the long chain aliphatics (e.g. tetradecane, pentadecane) penetrated in smaller amounts than shorter chain aliphatics.

Available data on the dermal absorption of individual hydrocarbons from petroleum products suggest that dermal exposure to these substances, even following long-term exposures such as in an occupational setting, will not cause systemic toxicity under normal working conditions and assuming an intact skin barrier. Furthermore, in most exposure scenarios, volatile hydrocarbons (such as aromatics) will easily evaporate from the skin, reducing dermal exposure and uptake. Dermal absorption of vapours can be considered negligible, as, even in the worst case scenario where total body exposure is assumed, dermal uptake will be much lower than respiratory uptake at identical air concentrations. Repeated skin contact with petroleum products may however lead to an impaired skin barrier and consequently enhanced uptake of hydrocarbons. To avoid this there is a need to minimise skin contact through use of protective clothing and gloves. For the purposes of quantitative risk assessment from dermal exposure, data on (or estimates of) external exposure (i.e. concentration of a hydrocarbon in petroleum product, duration of exposure, exposed skin area) and absorption rate of hydrocarbons are needed. In **Figure 6**, and **Tables IV** and **V**, a scheme for risk assessment of dermal exposure and the potential sources of data needed are presented.

7.2. LOCAL SKIN EFFECTS

Skin contact with certain petroleum substances or products may cause skin irritation, leading to dermatitis, particularly after repeated or prolonged exposure, which is assumed to be caused by aliphatic and aromatic hydrocarbons constituents. Aromatic solvents tend to be more irritant than aliphatic compounds. A number of petroleum hydrocarbons are classified as skin irritants, according to EU criteria.

In addition to the irritation effects of petroleum hydrocarbons, the skin barrier function may be affected following repeated contact, making the skin more susceptible to other irritants, sensitizing agents, and bacteria and also enhance the dermal penetration of other subtances. Furthermore, there is increasing evidence that severe, dermal irritation induced by long-term or repeated exposure to certain hydrocarbons can contribute to the progression-promotion effect and the development of skin tumours. The tumour-promoting activity of alkanes is related to their chain length, with maximal activity found in C12-C14 alkanes.

Petroleum hydrocarbons which have been tested for allergic contact dermatitis do not appear to be contact sensitising agents.

Figure 6 Health risk assessment of dermal exposure to petroleum hydrocarbons

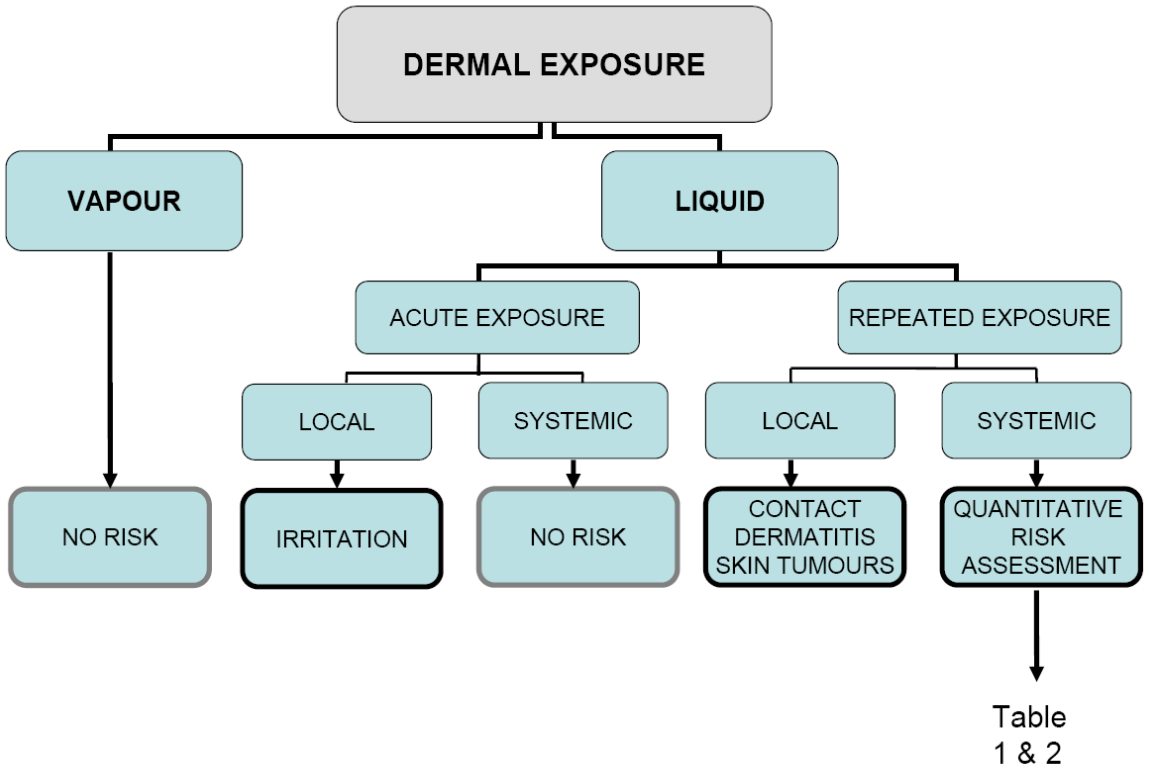
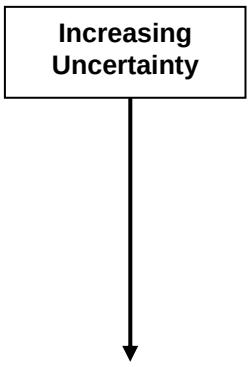


Table IV Tiered approach for data generation on skin absorption

Increasing Uncertainty ↓

Data on dermal absorption	**Predictive value**	**Remarks**
***In-vivo* human** Occupational studies Volunteer studies		Preferred data for risk assessment (gold standard)
***In-vivo* animal**	Pig skin is the best model animal for human skin Rat skin overestimates human dermal absorption	Application of rat skin data results in a conservative risk assessment
In-vitro Human or animal skin	Lower prediction value for highly lipophilic chemicals	When full thickness skin used, the amount of a chemical in all skin layers should be taken for the calculation Results obtained from dermal absorption studies with petroleum products are favoured above those carried out in aqueous solutions which overestimate dermal absorption
QSAR Skinperm Dermwin	Limited predictivity Not sufficiently validated	Kp significantly overpredicts dermal absorption of highly lipophilic petroleum products (log Kow>4) Maximal flux based on permeability coefficient, Kp and maximal solubility of a hydrocarbon in water gives better estimate than (Kp) alone Skinperm calculates dermal absorption to both, vapours and liquids. Furthermore it takes into account evaporation from the skin and calculates maximal flux based on maximum water solubility
Assumption 100% absorption		Conservative estimate

Table V Tiered approach for data generation on skin exposure



Source of data on dermal exposure	Remarks
Field studies **Experimental studies**	Limited standardisation and evaluation of the methods used
Exposure modelling **e.g.** **EASE** **DREAM** **RISKOFDERM**	Not sufficiently validated Most models do not take into account protective clothing and evaporation from the skin Most models show overprediction of exposure

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9. ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
AHC	Aliphatic hydrocarbons
AK	Adenylate kinase
ANN	Artificial neural network
ANOVA	Analysis of variance
AOEL	Acceptable operator exposure level
ASGDI	Atmospheric sampling glow discharge ionization
ATR-FTIR	Attenuated total reflectance-Fourier transform infrared spectroscopy
AUC	Area under the curve
BPD	Biocidal products directive
BSA	Bovine serum albumin
C	Carbon
CEFIC	European chemical Industry council/Conseil Européen de l'industrie chimique
CEN	The European committee for standardization
COLIPA	European cosmetic toiletry and perfumery association
D	Diffusion coefficient (cm^2/h)
DEO	Dermal exposure operation
DIEGME	Diethylene glycol-monomethyl ether
DMN	Dimethylbenzene
DMSO	Dimethysulfoxide
DOEL	Dermal occupational exposure limit
DREAM	Dermal exposure assessment method
EASE	Estimation and assessment of substance exposure
ECB	European chemical bureau
ECD	Electron capture detection
ECETOC	European centre for ecotoxicology and toxicology of chemicals
ECVAM	European Centre for Validation of Alternative Methods
EDETOX	Evaluation and Predictions of Dermal Absorption of Toxic Chemicals
EHC	Environmental health criteria
EPA (USA)	Environmental protection agency
ESAC	ECVAM Scientific Advisory Committee
ESR	Existing substances regulation
EUROPOEM	European predictive operator exposure model
FDA (USA)	Food and drug administration
FID	Flame ionization detection
FoD	Factor of difference
FPD	Flame photometric detection
FTIR	Fourier Transform Infra Red
GC	Gas chromatography
GHS	Globally harmonized system for the classification and labeling of chemical substances and mixtures
GPMT	The guinea pig maximization test
HEK	Human epidermal keratinocytes
HNTL	Highest non-cytotoxic level
HPLC	High performance liquid chromatography
HSE (UK)	Health and safety executive

ICH	International conference on harmonization
IL	Interleukin
iNOS	inducible nitric oxide synthase
IPPSF	Isolated perfused porcine skin flap
IR	Infrared
K	Partition coefficient
K_p	Permeability coefficient (cm/h)
LC	Langerhans cells
LLNA	Local lymph node assay
LMV	Low molecular weight
log K_{ow}	Lipophilicity of the petroleum hydrocarbons
LSD	Linear system dynamics
MS	Mass spectrometry
MW	Molecular weight
MTT	MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide]
NHEK	Normal human epidermal keratinocytes
OECD	Organization for economic cooperation and development
PAH	Polycyclic aromatic hydrocarbons
PBPK	Physiologically based pharmacokinetic model
PET	Pig Ear Test
P_{ow}	log K_{ow}
PPM	Part per million
RSD	Relative standard deviation
QSAR	Quantitative structure activity relationship
QSPeR	Quantitative structure permeability relationship
SAR	Structure activity relationship
SCCNFP	The scientific committee on cosmetic products and non-food products intended for consumers
SD	Standard deviation
SE	Standard error
SEM	Standard error of the mean
SIFT	The skin irritation function test
t_{lag}	Lag time (h)
TED	Thermal emission decay
TEM	Transmission electron microscopy
TER	The transcutaneous electrical resistance assay
TET	Tetradecane
TEWL	Trans-epidermal water loss
TMB	Trimethylbenzene
TNF-α	Tumor necrosis factor
UN	United Nations
UND	Undecane
USAF	United States Air Force

VITAE	Video imaging technique to assess exposure
VOC	Volatile organic compounds
WBC	White blood cells
WHO	World health organization

APPENDIX 1 SUMMARY DATA ON DERMAL ABSORPTION PARAMETERS FOR INDIVIDUAL HYDROCARBONS

Aliphatic hydrocarbons

Table 1: Hexane – summary					
Application of hexane	*In vivo/in vitro* animal-human	% dose absorbed	Flux ($mg/cm^2/h$)	Kp (cm/h)	Study
Hexane vapour	*In vivo*, rats		0.0065	0.031 ± 0.004	McDougal et al, 1990
Hexane vapour	*In vivo*, human volunteers			0.0051 ± 0.0036	Kezic et al, 2000

Table 2: Heptane – summary					
Application of heptane	*In vivo/in vitro* animal-human	% dose absorbed	Flux ($mg/cm^2/h$)	Kp (cm/h)	Study
Jet fuel (JP-8)	*In vivo* pigs	0.18			Singh et al., 2003
Jet fuel (JP-8)	*In vitro* human cadaver skin *In vitro* pig skin		x 10^{-6} 2.669 ± 0.577 4.540 ± 0.550	x 10^{-5} 10.65 ± 2.31 18.22 ± 2.20	Singh et al., 2002

Table 3: Nonane – summary					
Application of nonane	*In vivo/in vitro* animal-human	% dose absorbed	Flux ($mg/cm^2/h$)	Kp (cm/h)	Study
Jet fuel (JP-8)	*In vitro* human cadaver skin *In vitro* pig skin		x 10^{-3} 0.637 ± 0.058 0.477 ± 0.025	x 10^{-3} 0.0724 0.0541	Kanikkannan et al., 2001°
Jet fuel (JP-8)	*In vitro* rat skin		x 10^{-3} 0.384 ± 0.240	x 10^{-3} 0.042	McDougal et al, 2000
Jet fuel (JP-8)	*In vitro* pig skin After 1 day of pre-exposure Control Pre-exposed to jet fuel (JP-8) After 4 day of pre-exposure Control Pre-exposed to jet fuel (JP-8)		x 10^{-3} 0.03 ± 0.01 0.08 ± 0.01 0.03 ± 0.01 0.07 ± 0.01	x 10^{-3} 0.002 ± 0.001 0.005 ± 0.001 0.002 ± 0.000 0.005 ± 0.000	Muhammad et al, 2005
Jet fuel (JP-8)	*In vitro* pig ear skin JP-8 + 100 JP-8 + BHT JP-8 + MDA JP-8 + 8Q405		x 10^{-3} 0.395 ± 0.007 0.396 ± 0.014 0.451 ± 0.031 0.461 ± 0.033	x 10^{-4} 0.4489	Kanikkannan et al., 2001b

Table 4: Decane – summary					
Application of decane	*In vivo/in vitro* animal-human	% dose absorbed	Flux ($mg/cm^2/h$)	Kp (cm/h)	Study
Jet fuel (JP-8)	*In vivo* human volunteers			$x\ 10^{-5}$ 0.65 ± 0.33	Kim et al., 2006
Jet fuel (JP-8)	*In vitro* rat skin		$x\ 10^{-3}$ 1.65 ± 0.68	$x\ 10^{-3}$ 0.055	McDougal et al., 2000

Table 5: Undecane – summary					
Application of undecane	*In vivo/in vitro* animal-human	% dose absorbed	Flux ($mg/cm^2/h$)	Kp (cm/h)	Study
Jet fuel (JP-8)	*In vitro* pig skin dose levels: 1x dose 2x dose 5x dose		$x\ 10^{-3}$ 0.03 ± 0.00 0.03 ± 0.00 0.04 ± 0.01	$x\ 10^{-3}$ 0.0002 ± 0.0000 0.0002 ± 0.0000 0.0003 ± 0.0000	Muhammad et al., 2004
Jet fuel (JP-8)	*In vivo* human volunteers			$x\ 10^{-5}$ 0.045 ± 0.023	Kim et al., 2006
Jet fuel (JP-8)	*In vitro* rat skin		$x\ 10^{-3}$ 1.22 ± 0.81	$x\ 10^{-3}$ 0.025	McDougal et al., 2000
Jet fuel (JP-8)	*In vitro* pig skin After 1 day of pre-exposure Control Pre-exposed to jet fuel (JP-8) After 4 day of pre-exposure Control Pre-exposed to jet fuel (JP-8)		$x\ 10^{-3}$ 0.07 ± 0.01 0.16 ± 0.05 0.06 ± 0.01 0.10 ± 0.02	$x\ 10^{-3}$ 0.001 ± 0.000 0.003 ± 0.000 0.001 ± 0.000 0.002 ± 0.000	Muhammad et al., 2005

Table 6: Dodecane – summary					
Application of dodecane	*In vivo/in vitro* animal-human	% dose absorbed	Flux ($mg/cm^2/h$)	Kp (cm/h)	Study
Jet fuel (JP-8)	*In vitro* pig skin dose levels: 1x dose 2x dose 5x dose		$x\ 10^{-3}$ 0.01 ± 0.00 0.02 ± 0.00 0.03 ± 0.01	$x\ 10^{-3}$ 0.0003 ± 0.0001 0.0002 ± 0.0001 0.0001 ± 0.0000	Muhammad et al., 2004
Jet fuel (JP-8)	*In vivo* human volunteers			$x\ 10^{-5}$ 0.16 ± 0.056	Kim et al., 2006
Jet fuel (JP-8)	*In vitro* rat skin		$x\ 10^{-3}$ 0.510 ± 0.363	$x\ 10^{-3}$ 0.014	McDougal et al., 2000

Table 6: Dodecane – summary					
Various Jet fuels	*In vitro* pig skin Jet fuel (JP-8) Jet fuel (JP-A) Jet fuel (JP-8) puddle Jet fuel (JP-8(100))	 0.63 ± 0.04 0.29 ± 0.04 0.27 ± 0.07 0.35 ± 0.04			Riviere et al., 1999
Jet fuel (JP-8)	*In vitro* pig skin After 1 day of pre-exposure Control Pre-exposed to jet fuel (JP-8) After 4 day of pre-exposure Control Pre-exposed to jet fuel (JP-8)		x 10^{-3} 0.04 ± 0.01 0.06 ± 0.02 0.02 ± 0.00 0.05 ± 0.01	x 10^{-3} 0.0005 ± 0.000 0.0009 ± 0.000 0.0002 ± 0.000 0.0008 ± 0.000	Muhammad et al., 2005
Jet fuel (JP-8) with various additives	*In vitro* pig skin Jet-A Jet-A + DIEGME Jet-A + 8Q21 Jet-A + Stadis450 Jet-A + DIEGME + 8Q21 Jet-A + DIEGME + Stadis450 Jet-A + 8Q21 + Stadis450 JP-8		x 10^{-4} 0.10 ± 0.01 0.06 ± 0.003 0.05 ± 0.01 0.10 ± 0.01 0.19 ± 0.02 0.25 ± 0.05 0.30 ± 0.02 0.09 ± 0.01	x 10^{-4} 0.11 ± 0.01 0.09 ± 0.01 0.07 ± 0.01 0.15 ± 0.02 0.12 ± 0.01 0.15 ± 0.03 0.18 ± 0.01 0.09 ± 0.01	Baynes et al., 2001
Jet fuel (JP-8) with various additives	*In vitro* silastic membrane JP-8 (n = 5) JP-8 + MDA (n = 5) JP-8 + BHT (n = 5) JP-8 + 8Q405 (n = 4) JP-8 + MDA + BHT (n = 5) JP-8 + MDA + 8Q405 (n = 4) JP-8 + BHT + 8Q405 (n = 4) JP-8(100) (n = 5) *In vitro* pig skin JP-8 (n = 5) JP-8 + MDA (n = 5) JP-8 + BHT (n = 5) JP-8 + 8Q405 (n = 4) JP-8 + MDA + BHT (n = 5) JP-8 + MDA + 8Q405 (n = 4)		x 10^{-4} 1.46 ± 0.08 0.70 ± 0.03 0.90 ± 0.07 0.75 ± 0.04 0.89 ± 0.15 0.84 ± 0.08 0.74 ± 0.24 1.34 ± 0.05 0.090 ±0.01 0.164 ±0.03 0.123 ±0.01 0.171 ±0.05 0.077 ±0.01 0.097 ±0.01	x 10^{-4} 0.041 ±0.002 0.020 ±0.001 0.026 ±0.002 0.021 ±0.001 0.025 ±0.004 0.024 ±0.002 0.021 ±0.007 0.038 ±0.001 0.0025 ±0.00 0.0047 ±0.00 0.0035 ±0.00 0.0049 ±0.00 0.0022 ±0.00 0.0028 ±0.00	Muhammad et al., 2004

Table 6: Dodecane – summary					
	JP-8 + BHT + 8Q405 (n = 4) JP-8(100) (n = 5)		0.079 ±0.01 0.094 ±0.02	0.0022 ±0.00 0.0027 ±0.00	

Table 7: Tridecane – summary					
Application of tridecane	*In vivo/in vitro* animal-human	% dose absorbed	Flux ($mg/cm^2/h$)	Kp (cm/h)	Study
Jet fuel (JP-8)	*In vitro* pig skin dose levels: 1x dose 2x dose 5x dose		x 10^{-3} 0.004 ± 0.00 0.006 ± 0.00 0.008 ± 0.00	x 10^{-3} 0.0001 ± 0.000 0.0001 ± 0.000 0.0001 ± 0.000	Muhammad et al., 2004
Jet fuel (JP-8)	 *In vitro* human skin *In vitro* pig skin		x 10^{-3} 1.447 ± 0.154 1.508 ± 0.188	x 10^{-3} 0.0670 0.0698	Kanikkannan et al., 2001a
Jet fuel (JP-8)	*In vitro* rat skin		x 10^{-3} 0.334 ± 0.194	x 10^{-3} 0.015	McDougal et al., 2000
Jet fuel (JP-8)	*In vitro* pig skin After 1 day of pre-exposure Control Pre-exposed to jet fuel (JP-8) After 4 day of pre-exposure Control Pre-exposed to jet fuel (JP-8)		x 10^{-3} 0.02 ± 0.01 0.01 ± 0.00 0.00 ± 0.00 0.02 ± 0.01	x 10^{-3} 0.0003 ± 0.000 0.0002 ± 0.000 0.0001 ± 0.000 0.0003 ± 0.000	Muhammad et al., 2005
Jet fuel (JP-8)	*In vitro* pig ear skin JP-8 + 100 JP-8 + BHT JP-8 + MDA JP-8 + 8Q405		x 10^{-3} 1.318 ± 0.155 1.223 ± 0.059 1.530 ± 0.111 1.465 ± 0.093	x 10^{-4} 0.6102	Kanikkannan et al., 2001b

Table 8: Hexadecane – summary					
Application of hexadecane	*In vivo/in vitro* animal-human	% dose absorbed	Flux ($mg/cm^2/h$)	Kp (cm/h)	Study
Jet fuel (JP-8)	*In vivo* pigs	0.34			Singh et al., 2003
Jet fuel (JP-8)	*In vitro* pig skin Jet fuel (JP-8)	 0.18 ± 0.08			Riviere et al., 1999
Jet fuel (JP-8)	*In vitro* human cadaver skin *In vitro* pig skin		x 10^{-6} 1.586 ± 0.000 1.980 ± 0.000	x 10^{-5} 3.60 ± 0.00 4.60 ± 0.00	Singh et al., 2002

Table 9: Benzene – summary					
Application of benzene	*In vivo/in vitro* animal-human	% dose absorbed	Flux ($mg/cm^2/h$)	Kp (cm/h)	Study
Benzene vapour in air	*In vivo*, rats		0.0191	0.152 ± 0.006	McDougal et al, 1990
Benzene in water	*In vitro*, human Donor solution (26 ºC) Donor solution (50 ºC) Donor solution (40 ºC) Donor solution (15 ºC)			 0.14 ± 0.01 0.26 ± 0.05 0.18 ± 0.03 0.10 ± 0.04	Nakai et al, 1997
Benzene in water	*In vivo*, rats Previously frozen skin Skin pre-treated with moisturizer Skin pre-treated with baby oil Skin pre-treated with insect repellant Skin pre-treated with sunscreen			 0.18 ± 0.02 0.14 ± 0.03 0.16 ± 0.02 0.18 ± 0.02 0.24 ± 0.04	Nakai et al, 1997
Benzene in toluene Benzene in water	*In vitro* human skin Benzene in toluene (0.01 – 0.50 %) Receptor fluid Epidermis Dermis Benzene in water (0.10 – 0.50 %) Receptor fluid Epidermis Dermis	 0.08 – 0.12 0.03 – 0.04 0.01 – 0.01 3.88 – 5.03 0.36 – 0.42 0.27 – 0.41			Wester & Maibach, 2000
Benzene in different vehicles	*In vitro*, human Vehicle: Benzene Air Water Hexane Hexadecane Isooctane Gasoline		 1.861 ± 0.95 0.92 ± 0.33 0.194 ± 0.044 0.106 0.044 0.167 0.062	x 10^{-3} 111.1 ± 25.9 2.4 0.94 ± 0.38 3.73 ± 1.26 1.40 ± 0.58	Blank et al, 1985 Flux calculated using density of benzene to be 0.81 g/ml
Neat benzene	*In vitro*, rats		0.57		Ahaghotu et al, 2005
Gasoline	*In vitro*, human Gasoline 1 Gasoline 2	 0.49 0.63	x 10^{-3} 2.71 ± 1.62 1.80 ± 1.11	x 10^{-4} 4.95 6.35	Adami et al, 2006

Table 9: Benzene – summary					
	Gasoline 3 Mean ± SD	0.19 0.43 ± 0.23	1.47 ± 0.53 1.99 ± 0.64	1.88 4.34 ± 2.28	

Table 10: Ethylbenzene – summary					
Application of benzene	*In vivo/in vitro* animal-human	% dose absorbed	Flux ($mg/cm^2/h$)	Kp (cm/h)	Study
Jet fuel (JP-8)	*In vitro* pig skin After 1 day of pre-exosure Control Pre-exposed to jet fuel (JP-8) After 4 day of pre-exosure Control Pre-exposed to jet fuel (JP-8)		x 10^{-3} 1.04 ± 0.17 3.32 ± 0.52 0.61 ± 0.15 2.04 ± 0.17	x 10^{-3} 0.06 ± 0.009 0.19 ± 0.03 0.035 ± 0.009 0.12 ± 0.009	Muhammad et al., 2005

Table 11: Trimethylbenzene – summary					
Application of benzene	*In vivo/in vitro* animal-human	% dose absorbed	Flux ($mg/cm^2/h$)	Kp (cm/h)	Study
Jet fuel (JP-8)	*In vitro* pig skin After 1 day of pre-exosure Control Pre-exposed to jet fuel (JP-8) After 4 day of pre-exosure Control Pre-exposed to jet fuel (JP-8)		x 10^{-3} 1.01 ± 0.14 1.77 ± 0.21 0.49 ± 0.04 1.52 ± 0.10	x 10^{-3} 0.056 ± 0.008 0.10 ± 0.01 0.028 ± 0.002 0.09 ± 0.005	Muhammad et al., 2005

Table 12: Toluene – summary					
Application of toluene	*In vivo/in vitro* animal-human	% dose absorbed	Flux ($mg/cm^2/h$)	Kp (cm/h)	Study
Aqueous toluene	*In vivo*, human volunteers Dermal only			0.0115 ± 0.0074	Thrall et al, 2002
Neat toluene	*In vitro*, rats		0.38		Ahaghotu et al, 2005
Toluene vapour mixture	*In vivo*, rats		0.0206	0.721 ± 0.007	McDougal et al, 1990
Toluene	*In vivo*, guinea pigs	16.5			Boman et al, 1995
Toluene vapours	*In vivo*, human volunteers			0.050 ± 0.023	Kezic et al, 2000

Table 12: Toluene – summary					
Aqueous toluene	*In vivo*, rats	43.8 ± 9.6		0.074 ± 0.005	Thrall and Woodstock, 2002
Jet fuel (JP-8)	*In vitro*, rat skin		x 10^{-3} 0.535 ± 0.094	x 10^{-3} 1.1	McDougal et al, 2000
Jet fuel (JP-8)	*In vitro*, pig ear skin *In vitro*, human cadaver skin		x 10^{-3} 0.119 ± 0.004 0.095 ± 0.009	x 10^{-4} 2.47 1.97	Kanikkannan et al, 2001
Gasoline	*In vitro*, human Gasoline 1 Gasoline 2 Gasoline 3 Mean ± SD	 0.07 0.08 0.04 0.06 ± 0.02	x 10^{-3} 5.74 ± 2.77 3.60 ± 2.25 2.07 ± 1.11 3.80 ± 1.84	x 10^{-4} 0.722 0.82 0.403 0.648 ± 0.218	Adami et al, 2006
Jet fuel (JP-8)	*In vitro* pig ear skin JP-8 + 100 JP-8 + BHT JP-8 + MDA JP-8 + 8Q405		x 10^{-3} 0.094 ± 0.001 0.071 ± 0.013 0.114 ± 0.009 0.117 ± 0.005	x 10^{-4} 1.958	Kanikkannan et al., 2001b
Neat toluene	*In vivo* human volunteers		x 10^{-3} 11.64 ± 4.23		Kezic et al., 2001
Toluene radiolabeled	*In vivo*, albino hairless mice	15.4 ± 2.0	2.94 ± 2.27		Susten et al, 1990
Toluene in various solvents	*In vitro*, human Ventilation (ml/min) , neat toluene 0 90 400 900 Ventilation (ml/min) , Butanol 0 90 Ventilation (ml/min) Chloroform/MeOH 0 90	 2.3 ± 0.4 0.8 ± 0.2 0.5 ± 0.1 0.2 ± 0.03 6.6 ± 0.2 0.7 ± 0.3 3.1 ± 0.5 1.1 ± 0.3			Boman and Maibach, 2000
Toluene in various vehicles	*In vivo*, mice Vehicle: Toluene Methanol Ethanol 1-Propercutaneous absorptionnol			x 10^{-3} 0.0792 0.5904 0.1590 0.0714	Tsuruta et al, 1996

Table 12: Toluene – summary					
	Isobutanol			0.0714	
	1-Pentanol			0.0864	
	1-Ooctanol			0.0930	
	2-Metoxyethanol			0.0990	
	2-Butoxyethanol			0.1068	
	Benzyl alcohol			0.0834	
	Cyclohexanol			0.0768	
	Ethylene glycol			0.0564	
	Propylene glycol			0.1404	
	Glycerol			0.0864	
	Ether			0.1170	
	Acetone			0.1146	
	DMSO			0.714	
	N,N-Dimethylacetamide			0.3222	
	N,N-Dimethylformamide			0.5124	
	Benzene			0.0720	

Table 13: Xylene – summary					
Application of xylene	*In vivo/in vitro* animal-human	% dose absorbed	Flux ($mg/cm^2/h$)	Kp (cm/h)	Study
Xylene	*In vitro*, perfused pig ear				deLange et al, 1994
	Whole blood		0.0211 ± 0.0028		
	Blood-WBC		0.0188 ± 0.0021		
	Plasma		0.0954 ± 0.0191		
	Buffer + BSA		0.0143 ± 0.0035		
	Buffer – BSA		0.0024 ± 0.0006		
m-xylene vapour	*In vivo*, rats		0.0151	0.723 ± 0.003	McDougal et al, 1990
Aqueous	*In vivo*, rats			0.058 ± 0.009	Thrall and Woodstock, 2003
o-xylene	*In vivo*, human volunteers			0.005 ± 0.001	
m-xylene vapour	*In vivo*, human volunteers			0.025 ± 0.012	Kezic et al, 2000
Neat m-xylene	*In vivo*, human volunteers		x 10^{-3} 2.40 ± 0.89		Kezic et al, 2001
Jet fuel (JP-8)			x 10^{-6}	x 10^{-5}	Singh et al, 2002
	In vitro human cadaver skin		2.211 ± 0.021	8.33 ± 0.01	
	In vitro pig skin		2.569 ± 0312	9.68 ± 0.01	
Gasoline	*In vitro*, human		x 10^{-3}	x 10^{-4}	Adami et al, 2006
	Gasoline 1	0.01	1.01 ± 0.59	0.097	
	Gasoline 2	0.01	0.50 ± 0.25	0.051	

Table 13: Xylene – summary					
	Gasoline 3 Mean ± SD	0.01 0.008 ± 0.003	0.71 ± 0.56 2.38 ± 0.17	0.104 0.084 ± 0.029	
Jet fuel (JP-8)	*In vitro* pig skin After 1 day of pre-exosure Control Pre-exposed to jet fuel (JP-8) After 4 day of pre-exosure Control Pre-exposed to jet fuel (JP-8)		x 10^{-3} 1.47 ± 0.20 3.80 ± 0.61 1.02 ± 0.28 3.13 ± 0.15	x 10^{-3} 0.085 ± 0.011 0.218 ± 0.040 0.059 ± 0.016 0.180 ± 0.009	Muhammad et al., 2005
m-xylene vapour	*In vivo*, human volunteers 20 min exposure 45 min exposure 120 min exposure 180 min exposure		x 10^{-4} 0.34 ± 0.12 0.42 ± 0.14 0.59 ± 0.16 0.63 ± 0.14	 0.059 ± 0.016 0.063 ± 0.014	Kezic et al, 2004
Jet fuel (JP-8)	*In vivo* pigs	0.12			Singh et al., 2003
Jet fuel (JP-8)	*In vitro* rat skin		x 10^{-3} 0.795 ± 0.238	x 10^{-3} 0.17	McDougal et al., 2000
Neat xylene	*In vitro*, rats		0.22		Ahaghotu et al, 2005
m-xylene in ethanol	*In vivo*, rats m-xylene alone m-xylene + sandy soil m-xylene + clay soil	 0.23± 0.03 0.15 ± 0.03 0.26 ± 0.02			Skowronski et al, 1990

Table 14: Benzo[a]pyrene – summary					
Application of benzo[a]pyrene	*In vivo/in vitro* animal-human	% dose absorbed	Flux (mg/cm^2/h)	Kp (cm/h)	Study
Petroleum crude oil	*In vivo* rats crude oil crude oil in soil *In vitro* rat skin crude oil crude oil in soil	 35.3 ± 2.6 9.2 ± 1.2 38.0 ± 3.0 8.5 ± 1.0			Yang et al., 1989
Industrial coal-tar	*In vitro* pig ear skin		x 10^{-4} 0.008		Van Rooij et al.,
PAHs in contaminated soil	*In vitro* cadaver skin benzo[a]pyrene in soil at low dose benzo[a]pyrene in soil at medium dose		x 10^{-6} 0.025 0.19		Roy et al., 1998

Table 14: Benzo[a]pyrene – summary					
	benzo[a]pyrene in soil at high dose benzo[a]pyrene in soil extract at high dose		1.00 210		
Mixture of PAHs	*In vitro* monkey skin Lubrication oil Acetone			x 10^{-3} * 0.23 ± 0.20	Sartorelli et al., 1999
Coal dust with acetone	*In vitro* human cadaver skin	8.57 ± 3.67			Sartorelli et al., 2001
Native PAHs in Lampsblack in soil	*In vitro* human cadaver skin Concentration level (mg/kg) 38 111 135 632 817 915 1702		x 10^{-6} 0.05 ± 0.08 0.05 ± 0.06 0.10 ± 0.03 0.20 ± 0.08 0.30 ± 0.08 0.20 ± 0.08 0.30 ± 0.08		Stroo et al., 2005

Table 15: Pyrene – summary					
Application of pyrene	*In vivo/in vitro* animal-human	% dose absorbed	Flux ($mg/cm^2/h$)	Kp (cm/h)	Study
Industrial coal-tar	*In vitro* pig ear skin		x 10^{-4} 0.008		Van Rooij et al.,
Mixture of PAHs	*In vitro* monkey skin Lubrication oil Acetone			x 10^{-3} 0.17 ± 0.04 4.13 ± 4.36	Sartorelli et al., 1999
Coal dust with acetone	*In vitro* human cadaver skin	51.98 ± 14.97			Sartorelli et al., 2001

Table 16: Naphthalene – summary					
Application of naphthalene	*In vivo/in vitro* animal-human	% dose absorbed	Flux ($mg/cm^2/h$)	Kp (cm/h)	Study
Radiolabelled naphthalene	*In vivo* mice Naphthalene alone Naphthalenein sandy soil Naphthalene in clay soil	 0.50 ± 0.04 0.42 ± 0.03 0.63 ± 0.03			Turkall et al., 1994
Mixture of PAHs	*In vitro* monkey skin Lubrication oil acetone			x 10^{-3} 1.87 ± 1.31 6.31 ± 2.49	Sartorelli et al., 1999

Table 16: Naphthalene – summary					
Jet fuel (JP-8)	*In vivo* human volunteers			x 10^{-3} 0.053 ± 0.038	Kim et al., 2006
Mixture of aromatic and aliphatic hydrocarbons in hexadecane	*In vitro* pig skin Dose level: 1xdose 2xdose 3xdose		x 10^{-3} 0.43 ± 0.12 1.24 ± 0.26 3.63 ± 0.24	x 10^{-3} 0.033 ± 0.009 0.0485 ± 0.0101 0.0569 ± 0.0066	Muhammad et al., 2004
Jet fuel (JP-8)	*In vitro* pig skin		x 10^{-3} 0.376 ± 0.017	x 10^{-3} 0.181	Kanikkannan et al., 2001
Jet fuel (JP-8)	*In vitro* rat skin		x 10^{-3} 1.04 ± 0.38	x 10^{-3} 0.51	McDougal et al., 2000
Various jet fuels	*In vitro* porcine (pig) skin JP-8 Jet-A JP-8 (Puddle) JP-8 (100)	 1.17 ± 0.07 1.49 ± 0.18 1.11 ± 0.16 1.63 ± 0.29			Riviere et al., 1999
Jet fuel (JP-8) with various additives	*In vitro* porcine (pig) skin Jet-A Jet-A + DIEGME Jet-A + 8Q21 Jet-A + Stadis450 Jet-A + DIEGME + 8Q21 Jet-A + DIEGME + Stadis450 Jet-A + 8Q21 + Stadis450 JP-8		x 10^{-3} 0.222 ± 0.012 0.318 ± 0.036 0.264 ± 0.012 0.276 ± 0.012 0.124 ± 0.008 0.107 ± 0.014 0.097 ± 0.003 0.186 ± 006	x 10^{-3} 0.168 ± 0.006 0.198 ± 0.024 0.168 ± 0.006 0.174 ± 0.006 0.135 ± 0.010 0.113 ± 0.015 0.097 ± 0.003 0.144 ± 0.006	Baynes et al., 2001
Jet fuel (JP-8(100)) with various additives	*In vitro* pig skin and silastic membrane silastic membrane JP-8 (n = 5) JP-8 + MDA (n = 5) JP-8 + BHT (n = 5) JP-8 + 8Q405 (n = 4) JP-8 + MDA + BHT (n = 5) JP-8 + MDA + 8Q405 (n = 4) JP-8 + BHT + 8Q405 (n = 4) JP-8(100) (n = 5) pig skin JP-8 (n = 5) JP-8 + MDA (n = 5) JP-8 + BHT (n = 5) JP-8 + 8Q405 (n = 4)		x 10^{-3} 19.29 ± 0.60 28.23 ± 1.62 27.17 ± 0.84 26.86 ± 0.71 21.22 ± 0.52 21.65 ± 0.62 21.75 ± 0.73 21.01 ± 0.86 2.21 ± 0.27 2.63 ± 0.09 2.48 ± 0.28 2.17 ± 0.16 2.88 ± 0.35	x 10^{-3} 1.78 ± 0.06 2.30 ± 0.13 2.22 ± 0.07 2.19 ± 0.06 1.99 ± 0.05 2.03 ± 0.06 2.04 ± 0.07 1.93 ± 0.08 0.21 ± 0.03 0.20 ± 0.01 0.19 ± 0.02 0.17 ± 0.01 0.23 ± 0.03	Muhammad et al., 2004

Table 16: Naphthalene – summary					
	JP-8 + MDA + BHT (n = 5) JP-8 + MDA + 8Q405 (n = 4) JP-8 + BHT + 8Q405 (n = 4) JP-8(100) (n = 5)		2.64 ± 0.47 2.70 ± 0.48 2.24 ± 0.25	0.21 ± 0.04 0.21 ± 0.04 0.21 ± 0.02	
Jet fuel (JP-8)	*In vitro* pig skin After 1 day of pre-exposure Control Pre-exposed to jet fuel (JP-8) After 4 day of pre-exposure Control Pre-exposed to jet fuel (JP-8)		x 10^{-3} 4.19 ± 0.36 6.60 ± 0.79 4.81 ± 0.36 7.57 ± 0.47	x 10^{-3} 0.16 ± 0.01 0.26 ± 0.03 0.19 ± 0.01 0.29 ± 0.02	Muhammad et al., 2005
Jet fuel (JP-8)	*In vitro* pig ear skin JP-8 + 100 JP-8 + BHT JP-8 + MDA JP-8 + 8Q405		x 10^{-3} 0.419 ± 0.033 0.327 ± 0.015 0.386 ± 0.020 0.364 ± 0.037	x 10^{-4} 2.014	Kanikkannan et al., 2001b

Table 17: Methylnaphthalenes – summary					
Application of methylnaphthalene	*In vivo/in vitro* animal-human	% dose absorbed	Flux (mg/cm^2/h)	Kp (cm/h)	Study
Jet fuel (JP-8)	*In vivo* human volunteers 1-methylnaphthalene 2-methylnaphthalene			x 10^{-3} 0.029 ± 0.0059 0.032 ± 0.0074	Kim et al., 2006
Jet fuel (JP-8)	*In vitro* rat skin		x 10^{-3} 1.55 ± 0.52	x 10^{-3} 0.16	McDougal et al., 2000

Table 18: Dimethylnaphthalenes – summary					
Application of dimethylnaphthalene	*In vivo/in vitro* animal-human	% dose absorbed	Flux (mg/cm^2/h)	Kp (cm/h)	Study
Mixture of aromatic and aliphatic hydrocarbons in hexadecane	*In vitro* pig skin 1xdose 2xdose 3xdose		x 10^{-3} 0.13 ± 0.01 0.23 ± 0.05 0.58 ± 0.09	x 10^{-3} 0.0095 ± 0.0007 0.0088 ± 0.0020 0.0088 ± 0.0014	Muhammad et al., 2004
Jet fuel (JP-8)	In vitro rat skin		x 10^{-3} 0.586 ± 0.167	x 10^{-3} 0.93	McDougal et al., 2000
Jet fuel (JP-8)	*In vitro* pigs After 1 day of pre-exposure Control Pre-exposed to jet fuel (JP-8)		x 10^{-3} 0.62 ± 0.10 0.85 ± 0.21	x 10^{-3} 0.02 ± 0.004 0.03 ± 0.008	Muhammad et al., 2005

Table 18: Dimethylnaphthalenes – summary					
	After 4 day of pre-exposure Control Pre-exposed to jet fuel (JP-8)		 0.67 ± 0.07 0.99 ± 0.09	 0.03 ± 0.003 0.04 ± 0.004	

Table 19: Trimethylnaphthalenes – summary					
Application of trimethylnaphthalene	*In vivo/in vitro* animal-human	% dose absorbed	Flux ($mg/cm^2/h$)	Kp (cm/h)	Study
Jet fuel (JP-8)	*In vitro* rat skin		$x\ 10^{-3}$ 1.25 ± 0.50	$x\ 10^{-3}$ 0.13	McDougal et al., 2000

Table 20: Tetramethylbenzene – summary					
Application of pyrene tetramethylnaphthalene	*In vivo/in vitro* animal-human	% dose absorbed	Flux ($mg/cm^2/h$)	Kp (cm/h)	Study
neat	*In vitro* rat skin		< 0.02		Ahaghotu et al., 2005

APPENDIX 2 DETAILED DESCRIPTION OF EXPERIMENTAL CONDITIONS AND INTERPRETATION OF THE OUTCOMES

Table 1	Benzene
Authors	Blank *et al.*
Title	Penetration of benzene through human skin
Source	Journal of investigative dermatology, 85: 522–526, 1985

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* penetration through human skin from various vehicles Influence of pre-exposure to various vehicles on benzene permeation	-benzene -epidermal membrane of human abdominal skin static diffusion cells Analytical method: -GC analysis	-2-3 ml of solution containing benzene -vehicle: 1. pure benzene 2. hexadecane (50 µl/ml) 3. hexane (50 µl/ml) 4. gasoline (50 µl/ml) 5. isooctane (50 µl/ml) 6. water (2 µl/ml) 7. air (saturated) -duration of exposure: 3 h -exposure area: not clearly specified Permeation of benzene and ^{3}H water before and after pre-exposure to various solvents for three hours: -NaCl (0.1%) -Butanol -Hexane -Gasoline -Benzene Components measured: -Benzene -^{3}H water	Absorption parameters of benzene in various vehicles (mean or mean ± SD) Vehicle / Flux* (mg/cm^2/h) / Permeability coefficient* x 10^{-3} (cm/h) / Diffusion coefficient* x 10^{-5} (cm^2/h) / Partition coefficient Pure benzene: 1.86 ± 0.95 Air: 0.92 ± 0.33 Water: 0.194 ± 0.044 / 111.1 ± 25.9 / 1.48 / 30 Hexane: 0.106 / 2.4 / 6.84 / 0.14 Hexadecane: 0.044 / 0.94 ± 0.38 / 1.98 / 0.19 Isooctane: 0.167 / 3.73 ± 1.26 / 8.78 / 0.17 Gasoline: 0.062 / 1.40 ± 0.58 / 5.04 / 0.11 -gasoline (as a vehicle) seems to function most similarly to hexadecane - the *in vitro* system is assumed to measure the penetration parameters of benzene through hydrated *stratum corneum*. This may explain the higher values of the calculated flux than those obtained in *in vivo* situations. -The data showed that pre-exposure to very week salt caused little change in permeation of benzene and water, while butanol and hexane caused the skin to be more permeable to both molecules -Gasoline and benzene showed no influence on the benzene permeability, while the skin became permeable to water. It is suggested that different vehicles may alter the polar and non-polar pathways differently which is the cause of different permeabilities of benzene in various vehicles

<table>
<tr><td>Table 2</td><td colspan="3">Benzene, toluene, xylene, hexane</td></tr>
<tr><td>Authors</td><td colspan="3">McDougal et al.</td></tr>
<tr><td>Title</td><td colspan="3">Dermal absorption of organic chemical vapors in rats and humans</td></tr>
<tr><td>Source</td><td colspan="3">Fundamental and Applied Toxicology 14 :299–308, 1990</td></tr>
<tr><td>Type / aim of study</td><td>Test material/ species / technique / detection</td><td>Exposure condition</td><td>Results</td></tr>
<tr><td>- In vivoIn vivo assessment of whole body dermal permeation of aliphatic and aromatic hydrocarbons. Comparison with published human data on vapor penetration</td><td>- vapor of benzene, toluene, xylene and hexane

-male Fisher-344 rats (205-273 g)

Analytical method:
-GC-FID analysis</td><td>-whole body exposure to vapor mixture of chemicals using masks to prevent respiratory exposure

-exposure duration:
4 h

Components measured:
-m-xylene (22.03 g/m^3)
-toluene (30.57 g/m^3)
-benzene (129.63 g/m^3)
-hexane (214.61 g/m^3)</td><td>-The vapor permeability was analyzed using physiologically based pharmacokinetic modeling according to Ramsey & Andersen (1984)

<table><tr><th>Chemical</th><th>Flux (mg/cm^2/h)</th><th>Permeability coefficient (cm/h)</th><th>Skin uptake (%)</th></tr><tr><td>m-xylene</td><td>0.0151</td><td>0.723 ± 0.003</td><td>3.9</td></tr><tr><td>toluene</td><td>0.0206</td><td>0.721 ± 0.007</td><td>3.7</td></tr><tr><td>benzene</td><td>0.0191</td><td>0.152 ± 0.006</td><td>0.8</td></tr><tr><td>hexane</td><td>0.0065</td><td>0.031 ± 0.004</td><td>0.1</td></tr></table>
In general the results show that the permeability in rats is 2-4 times greater than in humans, based on literature data

Comment:
-The results for the individual compounds are calculated from the exposure to the mixture</td></tr>
</table>

<table>
<tr><td>Table 3</td><td colspan="3">Benzene, toluene, xylene, tetramethyl benzene</td></tr>
<tr><td>Authors</td><td colspan="3">Ahaghotu et al.</td></tr>
<tr><td>Title</td><td colspan="3">Effect of methyl substitution of benzene on the percutaneous absorption and skin irritation in hairless rats</td></tr>
<tr><td>Source</td><td colspan="3">Toxicology Letters 159: 261–271, 2005</td></tr>
<tr><td>Type / aim of study</td><td>Test material/ species / technique / detection</td><td>Exposure condition</td><td>Results</td></tr>
<tr><td>- In vitro assessment of dermal permeation rate and retention of benzene and methylbenzenes in the skin</td><td>kerosene aromatic hydrocarbons

-dorsal skin excised from hairless rats after euthanasia (removed adhering fat and subcutaneous tissue)

- Franz static diffusion cells

Analytical method:
-liquid scintillating counting</td><td>1 ml of pure substance spiked with radiolabeled compounds

Exposure area:
- 0.636 cm^2

Exposure duration:
- 8 h

Components measured:
-benzene
-toluene
-xylene
-tetramethyl benzene</td><td>Steady-state flux of neat aromatic hydrocarbons

<table><tr><td>Chemical</td><td>Steady-state Flux ($mg/cm^2/h$)</td></tr><tr><td>Benzene</td><td>0.57</td></tr><tr><td>Toluene</td><td>0.38</td></tr><tr><td>xylene</td><td>0.22</td></tr><tr><td>Tetramethyl benzene</td><td>< 0.02*</td></tr></table>*estimated from the Figure 1

- retention of all four chemicals in the stratum corneum is much higher than in epidermis and dermis at all time points (exact values are not available except for xylene, data are shown only graphically)
- higher retention of tetramethyl benzene than benzene in the stratum corneum showed effect of methyl substitution of benzene ring
- from the mass balance it was shown that for xylene the recovery ranged from 92 to 105%, indicating a minimum evaporation of xylene during the experiment

Comment:
-The data are presented only graphically, exact flux values are given for benzene, toluene and xylene, but corresponding standard deviations or standard error are not presented
-No flux values are presented for tetramethyl benzene, possibly since the flux (in the graph presented) is much lower than for benzene, toluene and xylene</td></tr>
</table>

Table 4	Benzene
Authors	Nakai *et al*.
Title	Effect of environmental conditions on the penetration of benzene through human skin
Source	Journal of Toxicolgy and Environmental Health 51: 447–462, 1997

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* penetration of benzene through human skin. Effect of various conditions associated with swimming and bathing	-Benzene -freshly prepared, dermatomed human abdomen and breast skin obtained from surgery (thickness 200-400 µm) Skin was also pre-treated with baby oil, skin moisturizer, sunscreen or insect repellent before the skin was dermatomed Analytical method: -liquid scintillation counting	400 ml of benzene in water containing radiolabelled benzene (C = 15-50 µg/l) Concentration of benzene was maintained constant by adding appropriate volume of stock of radiolabelled benzene spiked solution in methanol (< 20 µl) -duration of exposure: 8 h -exposure area: 0.2 cm^2 Components measured: -benzene	See table below

Condition Standard conditions	Cell Number of cells	Donor solution (µg/l)	K_p (cm/h)	K_p (cm/h) (Mean ± SD)
Donor solution (26 ºC)	6	18	0.15	0.14 ± 0.01
	6	19	0.14	
	6	47	0.13	
	6	47	0.14	
Donor solution (50 ± 2 ºC)	6	16	0.23	0.26 ± 0.05
	6	16	0.20	
	5	26	0.24	
	6	27	0.33	
	6	28	0.29	
Donor solution (40 ± 2 ºC)	4	17	0.19	0.18 ± 0.03
	4	23	0.20	
	6	28	0.13	
	6	30	0.21	
Donor solution (15 ± 2 ºC)	6	19	0.14	0.10 ± 0.04
	6	20	0.12	
	6	31	0.06	
	6	33	0.07	

-permeability was found to be concentration independent
-permeability coefficient of benzene through freshly prepared human skin was observed to increase with rising temperature
-there was a difference in permeability of a factor of 2.6 between highest and lowest temperature investigated

Condition	Number of cells	Donor solution (µg/l)	K_p (cm/h)	K_p (cm/h) (Mean ± SD)

Table 4	Benzene
Authors	Nakai *et al*.
Title	Effect of environmental conditions on the penetration of benzene through human skin
Source	Journal of Toxicolgy and Environmental Health 51: 447–462, 1997

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results

Previously frozen skin	6	18	0.19	0.18 ± 0.02
	6	19	0.19	
	6	33	0.16	
	6	35	0.16	
Skin pre-treated with moisturizer	6	16	0.14	0.14 ± 0.03
	5	21	0.09	
	6	31	0.15	
	6	37	0.16	
Skin pre-treated with baby oil	6	18	0.15	0.16 ± 0.02
	5	21	0.15	
	6	22	0.19	
	4	33	0.16	
Skin pre-treated with insect repellent	6	20	0.18	0.18 ± 0.02
	5	21	0.15	
	6	37	0.20	
	6	38	0.20	
Skin pre-treated with sunscreen	5	18	0.22	0.24 ± 0.04
	5	18	0.24	
	6	30	0.22	
	6	31	0.29	

-No significant difference was observed in permeability of benzene between untreated and pre-treated skin, except for skin pre-treated with sunscreen for which permeability coefficient of benzene was significantly higher than for non-treated skin

Table 5	Toluene			
Authors	Boman *et al*.			
Title	Percutaneous absorption of organic solvents during intermittent exposure in guinea pigs			
Source	Acta Dermato Venereologica, 75: 114–119, 1995			
Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results	Comment
-*In vivoIn vivo* assessment of percutaneous absorption of three solvents with various physico-chemical properties during brief intermittent exposure using an animal model	Test material: -toluene -n-butanol -1,1,1-trichloroethane Experimental method and species: - female guinea pigs (n = 5) Analytical method: -GC-FID	-neat toluene Intermittent exposure -duration of exposure: 8 x 1 min every 30 min -exposure area: 3.14 cm^2 Continuous exposure: -duration of exposure: 4 h -exposure area: 3.14 cm^2 Components measured: -toluene in blood	For the approximation of the total dose absorbed during the exposure, area under the time vs. blood concentration curve (AUC) was calculated for intermittent and continuous exposure to toluene The total dose absorbed of toluene was approximately 16.5% of continuous exposure (determined from graph, since the data were shown only graphically).	

Table 6	Toluene
Authors	Tsuruta
Title	Skin absorption of solvent mixtures-Effect of vehicles on skin absorption of toluene
Source	Industrial Health, 34: 369–378, 1996

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vivo*In vivo assessment of the effect of the vehicle on the skin permeation rate of toluene with various solvent mixtures	Test material: -toluene - in male mices (ICR, 30-40 g) (n = 5) -sacrifice at the end of exposure Analytical method: -GC-FID analysis	-0.5 ml of toluene in various solvents (50%, v/v) Intermittent exposure -duration of exposure: 8 x 1 min every 30 min -exposure area: 3.14 cm^2 Continuous exposure: -duration of exposure: 15, 30 and 60 min -exposure area: 3.14 cm^2 Components measured: -toluene in blood	Absorption parameters of toluene in various solvents (mean or mean ± SD)

Vehicle	Amount absorbed ($\mu g/cm^2$/30min)	K_p (cm/h) x 10^{-3}	Relative rate (toluene = 1)
Toluene	34.4 ± 9.1	0.0792	1
Methanol	128.0 ± 39.0	0.5904	7.5
Ethanol	34.4 ± 4.8	0.1590	2.0
1-Propanol	15.5 ± 1.0	0.0714	0.9
Isobutanol	15.5 ± 1.0	0.0714	0.9
1-Pentanol	18.7 ± 3.1	0.0864	1.1
1-Ooctanol	20.1 ± 3.5	0.0930	1.2
2-Metoxyethanol	21.5 ± 2.8	0.0990	1.3
2-Butoxyethanol	23.2 ± 8.9	0.1068	1.4
Benzyl alcohol	18.1 ± 2.8	0.0834	1.0
Cyclohexanol	16.6 ± 1.4	0.0768	1.0
Ethylene glycol	23.7 ± 4.5	0.0564	0.7
Propylene glycol	53.5 ± 16.0	0.1404	1.8
Glycerol	36.6 ± 19.0	0.0864	1.1
Ether	25.4 ± 8.9	0.1170	1.5
Acetone	24.8 ± 12.0	0.1146	1.5
DMSO	155.0 ± 30.0	0.714	9.0
N,N-Dimethylacetamide	69.8 ± 35.0	0.3222	4.1
N,N-Dimethylformamide	111.0 ± 62.1	0.5124	6.5
Benzene	15.6 ± 1.7	0.0720	0.9

-The data show that methanol, DMSO, N,N-Dimethylacetamide and N,N-Dimethylformamide act as strong penetration enhancers for toluene

-The absorbed amount of toluene increased as the ratio of methanol in the mixture increased which indicates that methanol enhances the skin absorption rate of toluene
-This is in contrast to mixtures of toluene and benzene, where increase of ratio of benzene in the mixture decreased the rate of toluene absorbed, while K_p remained about equal

Table 7	Toluene
Authors	Boman and Maibach
Title	Influence of evaporation and solvent mixtures on the absorption of toluene and n-butanol in human skin *in vitro*
Source	Annal of Occupational Hygiene, 44: 125–135, 2000

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* percutaneous absorption of toluene. Influence of forced ventilation	Test material: -toluene - human split-thickness skin (250 µm) flow through penetration-evaporation cell (adjustable ventilation above donor liquid) The evaporation rate was determined gravimetrically (Gilbert, 1971) Analytical method: -liquid scintillation counting	-radiolabelled toluene as neat toluene, toluene in butanol (50%, v/v), and toluene in chloroform, methanol and butanol (25:33.3:16.7:25, v/v) -applied volume: 200-300 µl -duration of exposure: 24 h -exposure area: 1.0 cm^2 Components measured: -toluene	Influence of ventilation air flow rate and cosolvent on *in vitro* skin absorption of toluene

Absorbed dose (% of total applied dose ± SD)					
Ventilation (Air flow, ml/min)	Donor 1	Donor 2		Donor 3	
	Neat toluene	Neat toluene	*CM	Neat toluene	**Butanol
0	1.9 ± 0.4	1.1 ± 0.3	3.1 ± 0.5	3.9 ± 0.5	6.6 ± 0.2
90	0.8 ± 0.2		1.1 ± 0.3		0.7 ± 0.3
400	0.5 ± 0.1				
900	0.2 ± 0.03				

*CM = 50/50 mixture with chloroform/methanol (2:1)
**Butanol = 50/50 mixture with butanol

-the absorption of toluene varied between donors in the absence of ventilation (0 ml/min)
-the absorption of toluene was increased when applied in mixtures, being highest in mixture with butanol at ventilation air flow rate of 0 ml/min
-at ventilation air flow rate of 90 ml/min there was a significant decrease of toluene absorption for all mixtures and
-ventilation decreased absorption of toluene

<table>
<tr><td>Table 8</td><td colspan="3">Toluene</td></tr>
<tr><td>Authors</td><td colspan="3">Thrall et al.</td></tr>
<tr><td>Title</td><td colspan="3">Use of real-time breath analysis and physiologically based pharmacokinetic modelling to evaluate dermal absorption of aqueous toluene in human volunteers</td></tr>
<tr><td>Source</td><td colspan="3">Toxicological Sciences, 68: 280–287, 2002</td></tr>
<tr><td>Type / aim of study</td><td>Test material/ species / technique / detection</td><td>Exposure condition</td><td>Results</td></tr>
<tr><td>-In vivoIn vivo assessment of the dermal absorption of aqueous solutions of toluene in humans under realistic exposure conditions

Comparison of uptake via dermal and inhalation exposure routes</td><td>-aqueous toluene
-human volunteers (n = 6)

Analysis using real-time exhaled breath analysis and PBPK modelling

Analytical method:
-GC-FID
-ASGDI-MS/MS</td><td>-volunteers were submerged to neck level into 397 l of tap water in a stainless steel hydrotherapy tub containing initially 500 µg/l of toluene

Volunteers were provided with purified breathing air

-duration of exposure:
20-30 min

-exposure area:
in range of 15060 to 22259 cm^2

Components measured:
-toluene in exhaled breath</td><td>PBPK model results for toluene exposure
<table>
<tr><th>Subject</th><th>Initial concentration in water (µg/l)</th><th>K_p (cm/h)</th></tr>
<tr><td>Volunteer 1</td><td>545.6</td><td>0.020</td></tr>
<tr><td>Volunteer 2</td><td>549.6</td><td>0.011</td></tr>
<tr><td>Volunteer 3</td><td>454.9</td><td>0.004</td></tr>
<tr><td>Volunteer 4</td><td>490.7</td><td>0.003</td></tr>
<tr><td>Volunteer 5</td><td>496.9</td><td>0.020</td></tr>
<tr><td>Volunteer 6</td><td>503.5</td><td>0.011</td></tr>
<tr><td>Mean ± SD</td><td>506.9 ± 35.8</td><td>0.0115 ± 0.0074</td></tr>
</table>
-Concentration profile of toluene vs. time in exhaled breath during exposure and some time after exposure showed that toluene is rapidly absorbed, with peak concentration achieved within seconds after addition of toluene to the water.
-upon exit from the water tub, the toluene concentration in exhaled breath decreased rapidly, being almost non-detectable at 5 minutes after the end of exposure.</td></tr>
</table>

<table>
<tr><td>Table 9</td><td colspan="3">Toluene</td></tr>
<tr><td>Authors</td><td colspan="3">Thrall and Woodstock</td></tr>
<tr><td>Title</td><td colspan="3">Evaluation of the dermal absorption of aqueous toluene in F344 rats using real-time breath analysis and physiologically based pharmacokinetic modelling</td></tr>
<tr><td>Source</td><td colspan="3">Journal of Toxicology and Environmental Health A, 65: 2087–2100, 2002</td></tr>
<tr><td>Type / aim of study</td><td>Test material/ species / technique / detection</td><td>Exposure condition</td><td>Results</td></tr>
<tr><td>-In vivoIn vivo assessment of the dermal absorption of toluene</td><td>-toluene

- male F344 rats (n = 3)

Analysis using real-time exhaled breath analysis and PBPK modelling

Analytical method:
-GC-FID
ASGDI-MS/MS</td><td>- approximately 2 ml of aqueous toluene was applied at two concentration levels: 0.5 mg/l and 0.25 mg/l

-duration of exposure:
5 h

-exposure area:
4.9 cm^2

Components measured:
-toluene in exhaled air (breath)</td><td>Dermal exposure of rats (mean ± SD, n = 3)
<table>
<tr><td></td><td>Exposure level (mg/kg bw)</td><td>K_p (cm/h)</td><td>% dose absorbed</td></tr>
<tr><td></td><td>1.75 ± 0.32
4.14 ± 0.38</td><td>0.076 ± 0.004
0.070 ± 0.004</td><td>45 ± 4
42 ± 18</td></tr>
<tr><td>*Mean ± SE</td><td></td><td>0.074 ± 0.005</td><td>43.8 ± 9.6</td></tr>
</table>
*average value of K_p and % dose absorbed between two concentration levels

-maximum peak concentration in exhaled breath was achieved within 1 h after the end of exposure at the high concentration level (0.5 mg/l), and similar results were obtained for the low concentration level (0.25 mg/l)
-both concentration-time profiles showed a slow elimination phase
-the data indicate rapid absorption of toluene through the rat skin</td></tr>
</table>

Table 10	Toluene
Authors	Klede *et al*.
Title	Transcutaneous penetration of toluene in rat skin a microdialysis study
Source	Experimental Dermatology, 14: 103–108, 2005

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vivoIn vivo* assessment of the permeation kinetics of toluene penetration after short and long exposure durations -Effect of exposure duration, tape stripping, and pre-treatment with barrier creams	-toluene - male Wistar rats (n = 82) Technique: microdialysis Analytical method: -GC-FPD -GC-ECD	-200 µl of neat toluene was applied Exposure duration: 15 min and 240 min Animals were sacrificed at the end of experiment (after 240 min for both exposures) Exposure area: 0.9 cm^2 Components measured: -Toluene in dialysate -o-cresol in urine (collected during 240 mins for both exposure durations)	Amount of toluene collected in the dialysate samples after dermal exposure (mean ± SEM)

Skin pre-treatment	µg (15 min exposure)	µg (240 min exposure)
Dialysate (AUC)		
Control	11.63 ± 1.54 (n = 8)	37.12 ± 2.14 (n = 7)
Control tape stripped	12.95 ± 1.54 (n = 8)	34.55 ± 7.27 (n = 8)
Cremor basalis control	12.18 ± 2.13 (n = 7)	40.36 ± 6.83 (n = 7)
Cremor basalis tape stripped	10.72 ± 3.41 (n = 6)	32.68 ± 9.92 (n = 7)
Arretil control	16.24 ± 3.97 (n = 6)	40.28 ± 6.05 (n = 6)
Arretil tape stripped	13.68 ± 2.84 (n = 7)	38.23 ± 8.50 (n = 5)
Urine (o-cresol)		
Control	8.4 ± 1.0	12.7 ± 1.4

-significant difference in AUC for dialysate content of toluene was observed between short and long exposure durations, but no difference was present among different pre-treatments.

-there was a significant difference in o-cresol content in urine after 15 min and 240 min exposure (ANOVA, $p < 0.001$), but according to the authors there was no significant difference between different pre-treatments (data were not reported)

-the urine o-cresol content reflected the findings of toluene content in dialysate for the different exposure durations although to a lesser extent (factor of difference of 1.5) in comparison to the toluene content found in the dialysate (average factor of difference of 3). This could be explained by a slower elimination process of toluene and incorporation of toluene in adipose tissue. The authors did not collect urine at later time points, which would probably have yielded more urine o-cresol and give more precise data on the effect of exposure durations.

Table 11	Xylene		
Authors	De Lange *et al*.		
Title	The rate of Percutaneous permeation of Xylene, measured using the perfused pig ear model, is dependent on the effective protein concentration in the perfused medium		
Source	Toxicology and Applied pharmacology, 127: 298–305, 1994		
Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* dermal permeation rate of xylene, determined with perfused pig ear model. Effect of perfusion media, protein content of perfusate and perfusion flow on permeation rate	-xylene -perfused pig ear Analytical method: GC-FID	-neat xylene was applied -perfusion media used -whole blood -Blood-WBC - plasma -Buffer + BSA -Buffer - BSA Exposure duration: 4 h Exposure area: 10 cm^2 Buffer: phosphate buffered saline with 1 mg/ml glucose and 20 unit/ml heparyne	Permeation rate of xylene in perfused pig ear (mean ± SEM, n = 6) Sample / Permeation rate ($mg/cm^2/h$) Whole blood: 0.0211 ± 0.0028 Blood-WBC: 0.0188 ± 0.0021 Plasma: 0.0954 ± 0.0191* Buffer + BSA: 0.0143 ± 0.0035 Buffer – BSA: 0.0024 ± 0.0006* *significantly different from whole blood ($p < 0.05$), WBC-white blood cells BSA-bovine serum albumin -the permeation rate decreased about 9-fold when Buffer only was used as a perfusate and increased about 4.5 times when Plasma as perfusate was used compared to whole blood as perfusate -according to the authors the data indicated that net amount of protein passing through pig ear was responsible for the differences in permeation rate -to test the hypothesis, the authors performed another study with varying perfusate flow and varying albumin concentration. -the permeation rate increased with increasing perfusate flow at constant albumin concentration (4.5%) reaching a maximum at about 0.7 ml/min -the permeation rate increased with increasing albumin concentration at constant perfusate flow (0.6 ml/min) reaching a maximum at about 4.5% albumin -It was concluded that the use of whole blood remains the preferred vehicle of choice

Table 12	Xylene
Authors	Thrall and Woodstock
Title	Evaluation of the dermal bioavailability of aqueous Xylene in F344 rats and human volunteers
Source	Journal of Toxicology and Environmental Health, Part A, 66: 1267–1281, 2003

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vivoIn vivo* assessment of the dermal absorption of aqueous o-xylene in rats using exhaled breath analysis and PBPK modelling -evaluation of species differences in the dermal bioavailability of o-xylene	-o-xylene -male F344 rats (n = 6) -human volunteers (n = 3) Analytical method: -GC-FID -ASGSI-MS/MS	F344 rats: - application of exposure patch containing 2 ml of aqueous o-xylene (200 µg/ml) -exposure duration: 4 h -exposure area: 2.27 cm^2 Human volunteers: (exposure to lower legs only in stainless steel hydrotherapy tub containing 322 l of tap water with o-xylene(500 µg/l) -exposure duration: 10-30 min -exposure area: 1503, 1971 and 1990 cm^2	Xylene exposure results of humans and permeability as derived by PBPK modeling

	Initial water concentration of o-xylene (µg/l)	Peak exhaled breath concentration (ppb)	Kp (cm/h)
Human volunteers (n = 3)			
Volunteer 1 (1990 cm^2)	481.2	75	0.004
Volunteer2 (1503 cm^2)	469.1	75	0.005
Volunteer 3 (1972 cm^2)	477.7	100	0.005
Average (mean ± SD)	476 ± 6.2		0.005 ± 0.001
F344 rats (n = 6)			
Average (mean ± SD)			0.058 ± 0.009

-the results indicated that aqueous xylene is rapidly absorbed through human and rat skin.
-approximately 23% of the aqueous xylene was absorbed during 4 h period of monitoring the animals
-permeability coefficient was about 12 times lower in humans then in rats

Comment:
-no data on exact information on individual exposure duration
-the authors stated that initial water concentration of o-xylene was about 400 times lower for rats than it was for humans

Table 13	Xylene
Authors	Kezic *et al*.
Title	Percutaneous absorption of m-xylene vapour in volunteers during pre-steady and steady state
Source	Toxicology Letters, 153: 273–282, 2004

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vivoIn vivo* assessment of percutaneous absorption of m-xylene vapour through human skin	-m-xylene -human male volunteers (n = 6) Data analysis: -by linear system dynamics (LSD, Opdam, 1991)using a reference exposure via inhalation and (de)convolution to yield dermal permeation rate time courses Analytical method: -GC-FID	Dermal exposure: -body part: arm in glass chamber of 60 cm in length) -exposure concentration: 29.4 mg/m^3 (range: 24.9 – 34.0 mg/m^3) -flow rate of xylene vapour: 3 l/min: -exposure duration: 20, 45, 120 and 180 min -exposure area: Average: 1178 cm^2 (range: 1100-1285 cm^2) Components measured: -xylene in exhaled breath	Maximum blood flux for different exposure durations

	Maximum flux x 10^{-5} (mg/cm^2/h)			
Exposure duration	20 min	45 min	120 min	180 min
Volunteer 1	3.6	4.1	5.7	6.9
Volunteer2	2.8	2.1	3.0	3.4
Volunteer 3	9.0	11.0	12.0	12.1
Volunteer 4	0.5	1.2	2.8	4.8
Volunteer 5	1.2	2.9	3.1	3.9
Volunteer 6	3.2	3.7	9.1	6.8
Average (mean ± SD)	3.4 ± 1.2	4.2 ± 1.4	*5.9 ± 1.6	*6.3 ± 1.4
	Apparent permeability coefficient (K_p, cm/h)			
Average Kp (mean ± SD)			*0.059 ± 0.016	*0.063 ± 0.014

*all fluxes were adjusted to an exposure concentration of 1 µg/cm^3 assuming a linear relationship between fluxes and concentration

-permeability coefficient was calculated only for 120 and 180 min exposure since after 90 min of exposure permeation rates reached a constant value indicating attainment of pseudo steady-state permeation

Table 14	Xylene and toluene		
Authors	Brooke *et al*.		
Title	Dermal uptake of solvents from the vapour phase: an experimental study in humans		
Source	Annals of Occupational Hygiene, 42: 531–540, 1998		
Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vivoIn vivo* dermal uptake of vapours containing xylene and toluene.	-xylene -toluene - human volunteers (males and females, four for each substance) Analytical method: -GC-MS -HPLC	Xylene – 100 ppm Toluene – 50 ppm Exposure: 1. Whole body exposure (dermal + inhalation exposure) 2. Dermal only exposure Volunteers wore air-fed masks to prevent inhalation exposure -exposure duration: 4 h Components measured: Xylene in blood and exhaled breath -2-methylhippuric acid in urine Toluene in blood and exhaled breath	Estimates of the dermal absorption of xylene and toluene (range)

	Whole body	Dermal only	% dermal only of the whole body exposure
Xylene			
Blood (µmol/l)	5.85 - 10.87	0.10 - 0.23	1.0 - 2.7
Breath (nmol/l)	401 - 676	5.2 – 18.5	0.7 – 4.6
#Urine (µmol)	1790 - 3716	27 - 43	0.7 – 1.6
Toluene			
Blood (µmol/l)	5.0 - 6.9	0.00 - 0.14	0 - 2
Breath (nmol/l)	385 - 598	5 - 10	0.9 – 2.6

*toluene was only found in the blood samples of two volunteers
#elimination via urine by assessing 2-methylhippuric acid

-vapours of toluene and xylene that were absorbed across the skin are estimated to provide a relatively small contribution to the total body burden after whole body exposure (approximately 1-3%)

Table 15	Xylene and toluene
Authors	Kezic *et al*.
Title	Skin absorption of some vaporous solvents in volunteers
Source	International Archive of Occupational and Environmental Health, 73: 415–422, 2000

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vivoIn vivo* assessment of percutaneous absorption rates of m-xylene, toluene and hexane vapour in humans	-m-xylene, toluene, hexane -human volunteers (n = 5) Data analysis: -by linear system dynamics (LSD, Opdam, 1991)using a reference exposure via inhalation and (de)convolution to yield dermal permeation rate time courses Analytical method: -GC-FID	Inhalation (reference) exposure: -exposure concentration: C< occup. exp. limit in NL -exposure duration: 10 min Dermal exposure: -body part: arm in glass chamber of 60 cm in length) -exposure concentration: m-xylene: 0.23 mmol/l toluene: 0.70 mmol/l hexane: 1.31 mmol/l (CV < 5%) -exposure duration: m-xylene: 20 min toluene: 20 min hexane: 30 min -exposure area: range: 960-1070 cm^2 Components measured: -xylene, toluene and hexane in exhaled air	Absorption rates into the skin ($RATE_{skin}$) and the maximum absorption rates into the blood ($RATE_{blood,\ max}$) (mean ± SEM) Component / $RATE_{skin}$ (cm/h) / $RATE_{blood,\ max}$ (cm/h) m-xylene / 0.12 ± 0.026 / 0.025 ± 0.012 toluene / 0.14 ± 0.055 / 0.050 ± 0.023 hexane / 0.013 ± 0.004 / 0.0051 ± 0.0036 Since the duration of exposure in this study was not long enough to reach steady-state, Kp could not be determined. However, since K_p is always lower than the absorption rate into the skin and higher than or equal to the maximum absorption rate into the blood, the values given in the table represent the lower and upper limits for $K_{p,}$ respectively Both the absorption rates into the skin and into the blood were similar for xylene and toluene and they were approximately 10-20 times higher than obtained for hexane.

Table 16	Toluene
Authors	Susten *et al*.
Title	*In vivoIn vivo* percutaneous absorption studies of volatile organic solvents in hairless mice II. Toluene, ethylbenzene and aniline
Source	Journal of applied Toxicology, 10: 217–225, 1990

Type / aim of study

-*In vivoIn vivo* percutaneous absorption of toluene and ethylbenzene in hairless mice

Test material/ species / technique / detection

- toluene, ethylbenzene

- male albino hairless mice (n = 5)

Analysis:
Mass balance

Analytical method:
-liquid scintillation counting

Exposure condition

-exposure concentration:
5 µl of treatment solution containing radiolabelled toluene or ethylbenzene

-exposure duration:
Toluene: 113 sec
Ethylbenzene: 294 sec

-exposure area:
0.8 cm^2

Components measured:
-toluene and ethylbenzene in expired breath, excreta and carcass

Results

Applied and absorbed amounts and absorption rates for toluene and ethylbenzene (mean ± SD)

Component	Amount applied (mg)	*Amount absorbed (mg)	Absorption rate ($mg/cm^2/h$)
Toluene	3.89 ± 0.25	0.0897 ± 0.0708	2.94 ± 2.27
ethylbenzene	4.10 ± 0.19	0.1486 ± 0.1278	2.22 ± 1.89

*amount absorbed = determined from radioactivity levels found in expired breath, excreta and carcass

Distribution of of the absorbed of ^{14}C labelled aromatic solvents after dermal exposure (% nominal dose recovered, mean ± SEM)

Component	Absorbed total	Carcass	Expired breath	Excreta
Toluene (n = 12)	15.4 ± 2.0	11.0 ± 3.0	20.5 ± 5.0	53.1 ± 6.0
Ethylbenzene (n = 11)	15.5 ± 2.0	4.5 ± 1.0	14.3 ± 6.0	65.6 ± 5.0

-the amount of ethylbenzene absorbed was greater due to the longer exposure duration than toluene, although toluene had a greater absorption rate.

Table 17	Xylene		
Authors	Skowronski *et al.*		
Title	Effects of soil on the dermal bioavailability of m-xylene in male rats		
Source	Environmental Research, 51: 182–193, 1990		
Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-Qualitative and quantitative *in vivoin vivo* assessment of the absorption, distribution, excretion and metabolism of soil adsorbed m-xylene versus pure m-xylene	-m-xylene - male Sprague-Dawley rats Analysis: Mass balance Analytical method: -liquid scintillation counting -HPLC	-Exposure concentration: 225 µl of ethanol solution containing radiolabelled m-xylene: alone or in combination with 750 mg of soil (surface concentration 15 mg m-xylene per cm^2) -Soil type: 1. Atsion sandy soil containing 90% sand, 2% clay and 4.4% organic matter 2. Keyport clay soil containing 50% of sand and 22 % of clay and 1.6% organic mater -Exposure duration: 72 h -Exposure area: 13 cm^2 Components measured: -radiolabeled m-xylene in excreta, blood, expired air and organs	Plasma half-lives and AUC plasma concentration time curves of radioactivity following dermal exposure to radiolabeled m-xylene (mean or mean ± SEM) Component / AUC (% initial dose/ml h) / $t_{1/2}$ / h absorption / $t_{1/2}$ / h elimination m-xylene alone: 0.23± 0.03 / 0.86 / 15.08 m-xylene + sandy soil: 0.15 ± 0.03 / 0.64 / 16.48 m-xylene + clay soil: 0.26 ± 0.02 / 2.40 / 15.43 The major urinary metabolite during 0-24 h collection period was methylhippuric acid (70-91%). Parent compound and metabolite xylenol were also detected in urine (0.4 – 3.8% and 2.6 -16.3%, respectively). There was no significant difference in excreta between different treatment groups. m-xylene derived-radioactivity after the pure and sandy soil treatment found in urine was around 42% while 62 to 67 % was found in expired air, respectively. Clay soil had somewhat higher excretion (46%) in urine, and lower amount was found in expired air (53%). The radioactivity found in faeces was negligible compared to urine and expired air (0.1 – 0.3%) for all three treatments. The stronger affinity of m-xylene for clay soil is supported by increased half-life of absorption. Furthermore, more radioactivity was found in fat tissue beneath the treated skin of the m-xylene clay soil group compared to m-xylene alone and m-xylene sandy soil groups.

Table 18	Xylene
Authors	Morgan *et al*.
Title	Dermal absorption of neat and aqueous volatile organic chemicals in the Fisher 344 rat
Source	Environmental Research, 55: 51–63, 1991

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vivoIn vivo* dermal absorption after exposure to neat and aqueous solutions of volatile organic chemicals (VOC). Effect of the degree of saturation	-various VOCs -male Fisher 344 rats Analytical method: -GC-FID -GC-ECD	-Exposure concentration: 2 ml of test compound was applied as neat, saturated, two-third saturated or one-third saturated aqueous solution -Exposure duration: 24 h -Exposure area: 3.1 cm^2 Components measured: -VOCs	Volumes of (aqueous solutions of) chemicals absorbed after 24 h dermal exposure (initial volume applied = 2 ml, mean ± SD)

Component	Neat (ml)	Saturated (ml)	Two-thirds saturated (ml)	One-third saturated (ml)
Benzene	0.62 ± 0.21	0.21 ± 0.10	0.26 ± 0.06	0.14 ± 0.00
Toluene	0.56 ± 0.37	0.27 ± 0.08	0.31 ± 0.21	0.68 ± 0.35
m-Xylene	0.65 ± 0.43	0.38 ± 0.20	0.51 ± 0.13	0.56 ± 0.41
ethyl benzene	0.24 ± 0.24	0.20 ± 0.10	0.18 ± 0.06	0.17 ± 0.07
n-hexane	0.98 ± 0.55	0.33 ± 0.08	0.14 ± 0.10	0.21 ± 0.09

*The volume of chemical absorbed was calculated by subtraction the volume remaining from the initial 2-ml volume.

The volumes of chemical absorbed varies between animals considerably and possible dependence of volume with concentration was not apparent

Neat chemical: After reaching the peak blood level within two hours, this peak level rapidly decreased to near control level except in the case of benzene where it continued to increase (data shown graphically only).
Aqueous chemical: Similar situation was observed for saturated solution of xylene and benzene and two-thirds saturated benzene, after reaching peak blood level within two hours it decreased rapidly to near control level. The data for other compound were not shown (data shown only graphically).

This pattern was attributed to the depletion of chemical in the donor solution and rapid distribution and elimination of chemicals. The depletion of chemical was supported by determination of concentration of VOCs left in the remaining volume of aqueous solution in exposure cell: the remaining volume contained less then 1 %of the initial VOC concentration .The evaporation of the chemical from the skin was prevented by teflon sealing caps found not to adsorb the chemicals.

Table 19	Xylene		
Authors	Chang *et al*.		
Title	Evaluation of dermal absorption and protective effectiveness of respirators for xylene in spray painters		
Source	Internatinal Archive ofor Occupational and Environmental health, 81(2): 145-150, 2007		
Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vivoIn vivo* determination of the contribution of dermal absorption on the total absorbed dose. Effectiveness of respirators in the field for xylene in spray painters	-xylene -ethyl benzene - male spray painters (n = 18) Analysis: Repeated-measures study design (collection of urine during 3-day work period before and after the work shift) Measurement of chemical concentration in the air Analytical method: -GC-FID	-Occupational exposure: Volunteers wore half-face respirators -Exposure duration: Repeated measurements 3-day work period of 8 h -Exposure area: Whole body Components measured: -methylhippuric acid and creatinine in urine	Estimation of urinary methylhippuric acid level coming from inhalation and dermal absorption (mg/g creatinine, mean ± SE) Component | Inhalation | **Dermal | Total | *Rate (%) Methylhippuric acid | 41.0 ± 4.98 | 202.1 ± 40.1 | 240.2 ± 42.3 | 63.7 ± 4.27 *Rate(%) = [(total – inhalation)/total] x 100%, **Dermal absorption = total - inhalation Concentration of xylene and ethylbenzene (highest exposure to those two chemicals) in ambient air was 52.6 ± 63.7 and 33.2 ± 32.4 ppm and the concentration of xylene and ethylbenzene inside the mask was 2.09 ± 2.74 and 1.79 ± 2.16 ppm (mean ± SD), respectively The authors estimated that the average fraction of the total absorption of xylene that is dermally absorbed was 63.7 ± 4.27 % and ranged from 27.3 to 92.3 %. Fraction of the total absorption of xylene that is dermally absorbed which was greater than 50% was observed in 14 of 18 spray painters. According to the authors, the inhalation exposure decreased due to wearing of protective respiratory mask and dermal exposure became the main contributor to the total body burden of solvents

Table 20	Jet fuel
Authors	Muhammad *et al*.
Title	Dose related absorption of JP-8 jet fuel hydrocarbons through porcine skin with quantitative structure permeability relationship analysis
Source	Toxicology Mechanisms and Methods, 14: 159–166, 2004

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* comparison of percutaneous absorption of various JP-8 hydrocarbons -assessment of the effect of dose on the *in vitro* percutaneous absorption of jet fuel (JP-8) hydrocarbons	-jet fuel (JP-8) aliphatic and aromatic hydrocarbons - flow through diffusion cells -dermatomated (200-300µm) perfused porcine skin from the dorsal area of weanling female Yorkshire pigs Analytical method: -GC-MS analysis -GC-FID analysis	-20 µl of mixture of aliphatic and aromatic hydrocarbons (see type of study) using hexadecane as diluent - three dose levels: 1x dose 2x dose 5x dose -duration of exposure: 5 h -exposure area: 0.64cm^2 Components measured: -naphthalene -dimethyl naphthalene -undecane -dodecane -tridecane -tetradecane -pentadecane -hexadecane	Absorption parameters for selected aromatic and aliphatic hydrocarbons (mean ± SEM) Naphthalene Flux x 10^{-3} (mg/cm^2/h) 0.43 ± 0.12 1.24 ± 0.26 3.63 ± 0.24 K_p x 10^{-3} (cm/h) 0.0333 ± 0.0090 0.0485 ± 0.0101 0.0569 ± 0.0066 Dimethylnaphthalene Flux x 10^{-3} (mg/cm^2/h) 0.13 ± 0.01 0.23 ± 0.05 0.58 ± 0.09 K_p x 10^{-3} (cm/h) 0.0095 ± 0.0007 0.0088 ± 0.0020 0.0088 ± 0.0014 Undecane Flux x 10^{-3} (mg/cm^2/h) 0.03 ± 0.00 0.03 ± 0.00 0.04 ± 0.01 K_p x 10^{-3} (cm/h) 0.0002 ± 0.0000 0.0002 ± 0.0000 0.0003 ± 0.0000 Dodecane Flux x 10^{-3} (mg/cm^2/h) 0.01 ± 0.00 0.02 ± 0.00 0.03 ± 0.01 K_p x 10^{-3} (cm/h) 0.0003 ± 0.0001 0.0002 ± 0.0001 0.0001 ± 0.0000 Tridecane Flux x 10^{-3} (mg/cm^2/h) 0.004 ± 0.00 0.006 ± 0.00 0.008 ± 0.00 K_p x 10^{-3} (cm/h) 0.0001 ± 0.000 0.0001 ± 0.000 0.0001 ± 0.000 A dose dependent increase in absorption of aromatic hydrocarbons was demonstrated by concentration-time profile and calculated flux for naphthalene and dimethylnaphthalene. There was a significant difference between 5X dose and 1x/2x doses for concentration and flux of aromatic hydrocarbons. There was no significant difference between doses for permeability, diffusivity and percent dose. There were no significant differences among 1x, 2x and 5x doses regarding all determined parameter values; flux, permeability, diffusivity and percent dose for aliphatic hydrocarbons. ***Overall:*** The flux, permeability, and percent dose absorbed decreased with increasing logK_{ow}. Since the concentrations of tetradecane in the receptor fluid were below the LOD at low doses and pentadecane data showed high backgrounds, these data were not included.

Table 21	Kerosene		
Authors	Tsujino *et al*.		
Title	Distribution of kerosene components in rats following dermal exposure		
Source	International Journal of Legal Medicine 116 :207–211, 2002		
Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vivoIn vivo* evaluation of the tissue distribution of kerosene components in rats following dermal exposure	-standard kerosene: aliphatic and aromatic hydrocarbons - male Wistar rats (250-300 g) Analytical method: -GC-MS analysis	Rats were exposed for 1 h abdominally using 2x2 cm^2 cotton piece soaked with 1 ml of standard kerosene and sacrificed immediately after the end of exposure without post-exposure sampling -rats were sacrificed at 0-12 h post-exposure -exposure duration: 1 h Components measured: -aliphatic hydrocarbons C_9-C_{16} -aromatic hydrocarbons cumene pseudocumene mesitylene 1,2,3-trimethylbenzene	Experiment 1: -the data were presented as ratios of peak areas of aliphatic and aromatic hydrocarbons to analytical internal standard (IS) in each blood, brain, lung, liver spleen, kidney, muscle and adipose tissue -Ratios of aliphatic hydrocarbons to IS were significantly lower in kidney, liver and brain ($p = 0.002$, 0.01 and 0.05, respectively) compared to ratios of aromatic hydrocarbons to IS. Ratios of aliphatic hydrocarbons to is compared to ratios of aromatic hydrocarbons to IS were non-significantly lower in blood, spleen and the lungs ($p > 0.05$) while the ratios of aliphatic hydrocarbons to IS were non-significantly higher in muscle and adipose tissue than ratios of aromatic hydrocarbons to IS ($p > 0.3$). Overall, ratios of aliphatic hydrocarbons to IS (0.12 ±0.02) were significantly lowerr than ratios of aromatic hydrocarbons to IS (0.21 ±0.05) (mean ± SE, $p = 0.04$) These data showed that aromatic hydrocarbons were dermally absorbed to a greater degree than aliphatic hydrocarbons Comment: -not all data are shown -only three animals were used

Table 22	Kerosene
Authors	Tsujino *et al*.
Title	Dermal absorption of kerosene components in rats and the influence of its amount and area of exposure
Source	Forensic Science International 133: 141–145, 2003

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* and *in vivoin vivo* dermal permeation of kerosene -assessment of the relationship between the amount or the area of dermal exposure and the kerosene levels in biological samples. -determination of the minimal time necessary to detect kerosene components in biological samples following dermal exposure	-kerosene aliphatic hydrocarbons (AHC) and aromatic hydrocarbons (trimethylbenzenes-TMB) - male Sprague-Dawley rats (320-410 g) - static diffusion cells -clipped skin from the backs of six intact anesthetized rats Analytical method: -GC-MS analysis	*in vivo* exposure: -rats were exposed abdominally using different size of cotton piece soaked with variable volume of standard kerosene. Groups: 1 4 cm^2/1 ml 2 4 cm^2/4 ml 3 16 cm^2/4 ml 4 64 cm^2/16 ml -exposure duration: 2 h -blood samples were collected before, during and up to 90 minutes after the end of exposure *in vitro* exposure: -exposed area: 1.77cm^2 Two doses levels: - 30 µl and 120 µl -exposure duration: 24 h Components measured: -AHC: C_9-C_{16} -TMB 1,2,3-trimethylbenzene 1,2,4-trimethylbenzene 1,3,5-trimethylbenzene	-the *in vivo* results were presented as total levels of aliphatic or aromatic hydrocarbons in blood and exposed skin area and the *in vitro* results as levels in receptor fluid and exposed skin *In vivo exposure:* Time course changes in the total concentrations of three TMBs in blood were significantly different between groups 1 and 2, 1and 3 and 4 in respect to group 1, 2 and 3. There was no difference between groups 2 and 3. Kerosene levels 2 h following dermal exposure (mean ± SE)

Exposure	Blood (µg/g)		Exposed skin (µg/g)	
Group no.	TMB	AHC	TMB	AHC
2	0.08 ± 0.02	0.03 ± 0.01	4.56 ± 0.86	154.9 ± 62.3
3	0.09 ± 0.02	0.02 ± 0.01	4.61 ± 0.67	78.9 ± 30.5

In vitro exposure:
Kerosene levels after 24 h in the skin (mean ± SE)

Dose level	Receptor fluid (µg/g)		Exposed skin (µg/g)	
	TMB	AHC	TMB	AHC
30 µl	0.09 ± 0.01	0.04 ± 0.01	1.48 ± 0.19	310.7 ± 48.2
120 µl	0.33 ± 0.06	0.14 ± 0.06	7.65 ± 2.59	573.7 ± 107.0

TMB=trimethylbenzenes, AHC-aliphatic hydrocarbons

Comment:
The data were not shown fully for all groups investigated. The author concluded that the absorption of TMBs was influenced by total amount applied rather than exposure surface area. At the same time concentration of AHCs in the skin was dependent on the amount applied per unit area. The study results suggest that AHCs tended to stay in the skin *in vivo* and *in vitro* although to a lesser degree *in vivo*. There was no data on absolute or relative composition of AHC and TMB in the applied kerosene

Table 23	Kerosene		
Authors	Hieda *et al*.		
Title	Skin analysis following dermal exposure to kerosene in rats: the effects of postmortem exposure and fire		
Source	International Journal of Legal Medicine 118: 41–46, 2004		
Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vivo* dermal absorption of kerosene in rats. Evaluation of usefulness of skin analysis for the forensic examination of cases involving postmortem dermal exposure to kerosene and/or fire	-kerosene aliphatic and aromatic hydrocarbons -*in vivo*, male Sprague-Dawley rats (330-387 g) -ante-mortem and post-mortem exposure -components measured: -aliphatic hydrocarbons C_9-C_{16} -aromatic hydrocarbons 1,2,3-trimethylbenzene 1,2,4-trimethylbenzene 1,3,5-trimethylbenzene Analytical method: -GC-MS analysis	-rats (ante-mortem) were exposed abdominally or on the back using cotton piece soaked with 4 ml of standard kerosene. -after sacrificing, the trunk blood was collected -rats (post-mortem) were exposed abdominally or on the back using cotton piece soaked with 4 ml of standard kerosene. -exposure duration: 30 mins -part of the exposed skin (ante- and post-mortem) was burned with a portable burner	-the concentration of aliphatic and aromatic hydrocarbons was consistently lower in back skin than in abdominal skin for both ante- and post-mortem exposure -there was no difference in kerosene levels between ante-mortem and post-mortem exposure on abdominal skin and back skin the kerosene concentration in mildly and severely burned skin was 84% and 28% of that in non-burned exposed skin Comment: Authors suggested that the difference in kerosene levels between abdominal and back skin could be due to thickness difference in skin layers

Table 24	Kerosene		
Authors	Fujihara *et al*.		
Title	The levels of kerosene components in biological samples after repeated dermal exposure to kerosene in rats		
Source	Legal Medicine 6: 109–116, 2004		
Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
In vivo dermal exposure of rats to kerosene. Evaluation of accumulation of kerosene components after repeated (daily) dermal exposure	Standard kerosene aliphatic and aromatic hydrocarbons male Sprague-Dawley rats (332-450 g) Analytical method: GC-MS	-rats (divided into groups) were repeatedly exposed on the abdominal skin using cotton piece soaked with 4 ml of standard kerosene. -exposure duration: Single exposure for 1 h and sacrificed at 0 and 24 h after the end of exposure Repeated exposure for 5 consecutive days for 1 h each day and sacrificed at 0 and 24 h after the end of exposure period Components measured: -aliphatic hydrocarbons C_9-C_{16} -aromatic hydrocarbons 1,2,3-trimethylbenzene 1,2,4-trimethylbenzene 1,3,5-trimethylbenzene	Blood: There was no significant difference in the blood levels of aromatic hydrocarbons between groups which were sacrificed immediately after the end of exposure or between groups which were sacrificed at 24 h after the end of exposure, but there was a significant difference between groups sacrificed immediately and 24 h after the end of exposure being higher in the groups sacrificed immediately after the end of exposure There were no significant differences in the blood level of aliphatic hydrocarbons between all groups Skin: The skin level of aromatic hydrocarbons was lower then the level of aliphatic hydrocarbons in all groups regardless of repeated or single dose. Significantly high levels of aliphatic hydrocarbons were detected after the fifth exposure and immediate sacrificing of the animals compared to repeated exposure and sacrificing after 24 h and single dose and sacrificing at 0 and 24 h. Comment: The data were presented only graphically as total levels of aliphatic or aromatic hydrocarbons

Table 25	Jet fuel
Authors	Kanikkannan *et al*.
Title	Percutaneous absorption and skin irritation of JP-8 (jet fuel)
Source	Toxicology 161: 1-11, 2001

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* percutaneous absorption of JP-8	-jet fuel (JP-8), containing aliphatic and aromatic hydrocarbons - Franz diffusion cells - dermatomated (500 µm) pig ear skin -dermatomated (500 µm) human cadaver skin Analytical method: -liquid scintillation counting	-1 ml of JP-8 spiked with radiolabeled tridecane, nonane, naphthalene or toluene -duration of exposure: 24 h -exposure area: 1.1 cm^2 Components measured: -nonane -tridecane -toluene -naphthalene	Steady state flux and permeability coefficient of aliphatic and aromatic hydrocarbons from pig ear skin and human cadaver skin (mean ± SD)

Pig ear skin	% (w/w) in JP-8	Steady-state flux x 10^{-3} ($mg/cm^2/h$)	K_p x 10^{-4} (cm/h)
Tridecane	2.7	1.508 ± 0.188	0.698
Nonane	1.1	0.477 ± 0.025	0.541
Naphthalene	0.26	0.376 ± 0.017	1.81
toluene	0.06	0.119 ± 0.004	2.47
Human cadaver skin			
Tridecane	2.7	1.447 ± 0.154	0.670
Nonane	1.1	0.637 ± 0.058	0.724
Naphthalene	0.26	0.451 ± 0.022	2.17
toluene	0.06	0.095 ± 0.009	1.97

Steady state flux increased with increasing proportion of a chemical in JP-8 in both pig ear and human cadaver skin.

Factor of difference (FoD) expressed as steady state flux through pig ear skin divided by steady state flux through human skin was 1.042 for tridecane, 0.748 for nonane, 0.833 for naphthalene and 1.256 for toluene.
Only the difference between steady state flux values of tridecane in pig ear and human skin was not significant

All analyzed chemicals from JP-8 permeated significantly across pig ear skin and human skin without any apparent lag time.

It was stated that when the percentage of dose absorbed was plotted against time, the absorption profile of naphthalene was higher than that of tridecane (no profiles presented).

<table>
<tr><td>Table 26</td><td colspan="3">Jet fuel</td></tr>
<tr><td>Authors</td><td colspan="3">Singh et al.</td></tr>
<tr><td>Title</td><td colspan="3">In vivo percutaneous absorption, skin barrier perturbation and irritation from JP-8 jet fuel components</td></tr>
<tr><td>Source</td><td colspan="3">Drug and chemical toxicology, 26: 135–146, 2003</td></tr>
<tr><td>Type / aim of study</td><td>Test material/ species / technique / detection</td><td>Exposure condition</td><td>Results</td></tr>
<tr><td>-In vivo percutaneous absorption of three components of JP-8

Assesment of skin barrier pertubation and irritation by JP-8 components.</td><td>-jet fuel (JP-8) aliphatic and aromatic hydrocarbons

- weanling pigs

Analytical method:
-liquid scintillation counting</td><td>-0.5 ml of JP-8 spiked with 1 µmol of each radiolabelled component: heptane, hexadecane and xylene

-duration of exposure:
30 min

-exposure area:
3.14 cm^2

Components measured:
-heptane
-hexadecane
-xylene</td><td>Percentage of the applied dose absorbed into the skin

<table><tr><td>Component</td><td>% absorbed dose</td></tr><tr><td>Xylene</td><td>0.12</td></tr><tr><td>Heptane</td><td>0.18</td></tr><tr><td>Hexadecane</td><td>0.34</td></tr></table>
The percentage of the applied dose absorbed into the skin was greater for aliphatic heptane and hexadecane then aromatic xylene.</td></tr>
</table>

<table>
<tr><td>Table 27</td><td colspan="3">Jet fuel</td></tr>
<tr><td>Authors</td><td colspan="3">Kim et al.</td></tr>
<tr><td>Title</td><td colspan="3">Dermal absorption and penetration of jet fuel components in humans</td></tr>
<tr><td>Source</td><td colspan="3">Toxicology Letters, 165: 11–21, 2006</td></tr>
<tr><td>Type / aim of study</td><td>Test material/ species / technique / detection</td><td>Exposure condition</td><td>Results</td></tr>
<tr><td>-In vivo absorption and penetration of aromatic and aliphatic components of JP-8 in humans</td><td>-jet fuel (JP-8) aliphatic and aromatic hydrocarbons

- human adult volunteers (5 males and 5 females)

- forearm

Analytical method:
-GC-PID analysis
-GC-MS analysis</td><td>-0.5 ml of neat JP-8

-duration of exposure:
30 min

-exposure area:
2 x 10 cm^2

-after the end of exposure the exposed area was tape-stripped 10 times

Components measured:
- naphthalene
- 1-methyl naphthalene
- 2-methyl naphthalene
- undecane
- dodecane
- dodecane</td><td>Blood

Concentration and apparent permeability coefficient of aromatic and aliphatic hydrocarbons (mean ± SD)
<table><tr><th>Component</th><th>C(mg/ml)</th><th>K_p x 10^{-5} (cm/h)</th></tr><tr><td>Naphthalene</td><td>3.0</td><td>5.3 ± 3.8</td></tr><tr><td>1-methylnaphthalene</td><td>2.5</td><td>2.9 ± 0.59</td></tr><tr><td>2-methylnaphthalene</td><td>2.1</td><td>3.2 ± 0.74</td></tr><tr><td>Decane</td><td>28.3</td><td>0.65 ± 0.33</td></tr><tr><td>Undecane</td><td>111.2</td><td>0.045 ± 0.023</td></tr><tr><td>Dodecane</td><td>102.9</td><td>0.16 ± 0.056</td></tr></table>
-The flux of the aliphatic hydrocarbons was greater than the flux of aromatic hydrocarbons (concentration of the aliphatics in JP-8 was more than one order of magnitude greater then the concentration of the aromatics)
No (significant) lag times could be determined. The apparent permeability coefficient (K_p, cm/h) was decreasing in order: naphthalene >1-methyl naphthalene = 2-methyl naphthalene > decane > dodecane > undecane, revealing relatively higher absorption of aromatic than aliphatic hydrocarbons.

Tape striping
Tape stripping was done additionally only to better understand the absorption of jet fuel JP-8.
Mass per cm^2 decreased rapidly with successive tape strips (with increasing stratum corneum depth). Higher mass per cm^2 was found for aliphatic then for aromatic hydrocarbons in tape strips except for decane for which mass per cm^2 was similar to aromatic hydrocarbons</td></tr>
</table>

Table 28	Jet fuel
Authors	McDougal *et al*.
Title	Assessment of skin absorption and penetration of JP-8 jet fuel and its components
Source	Toxicological Sciences, 55: 247–255, 2000

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results	Comment
-*In vitro* dermal penetration and absorption of JP-8 and its major constituents	-jet fuel (JP-8) aliphatic and aromatic hydrocarbons - static diffusion cells -dermatomated (560 µm) male rat skin from the back area Analytical method: -GC-FID analysis -GC-MSD analysis	-neat JP-8 -duration of exposure: 3.5 h exposure was used for determination of aliphatic components in the skin and 4 h exposure was used for the determination of flux and K_p -exposure area: 4.9 cm^2 Components measured: -naphthalene -methyl naphthalenes -dimethyl naphthalenes -xylene -toluene -trimethylbenzene -ethylbenzene -undecane -dodecane -decane -tridecane -nonane -diethylene glycol-monomethyl ether (DIEGME)	Measured flux and permeability coefficient for aliphatic and aromativ hydrocarbons (mean or mean ± SD) Components found in the skin after 3.5 h of dermal exposure to JP-8 (mean or mean ± SD) R= ratio between mass in the skin and C (in JP-8)	For the chemicals with lower log K_{ow} the K_p was larger than for chemicals with a higher logK_{ow} value The data showed that the absorption of aromatic hydrocarbons is relatively higher than absorption of aliphatic hydrocarbons Only six aliphatic components could be identified in the skin (consisting of the *stratum corneum*, epidermis and part of the dermis) -in the experiment it was not possible to determine the contribution of each individual skin layer -

Measured flux and permeability coefficient for aliphatic and aromativ hydrocarbons (mean or mean ± SD)

Component	Flux ± SD x 10^{-3} ($mg/cm^2/h$)	K_p (cm/h)
naphthalene	1.04 ± 0.38	5.1×10^{-4}
methyl naphthalenes	1.55 ± 0.52	1.6×10^{-4}
dimethyl naphthalenes	0.586 ± 0.167	9.3×10^{-4}
xylene	0.795 ± 0.238	1.7×10^{-4}
toluene	0.535 ± 0.094	1.1×10^{-3}
trimethylbenzene	1.25 ± 0.50	1.3×10^{-4}
ethylbenzene	0.377 ± 0.146	3.1×10^{-4}
undecane	1.22 ± 0.81	2.5×10^{-5}
dodecane	0.510 ± 0.363	1.4×10^{-5}
decane	1.65 ± 0.68	5.5×10^{-5}
tridecane	0.334 ± 0.194	1.5×10^{-5}
nonane	0.384 ± 0.240	4.2×10^{-5}
DIEGME	51.5 ± 15.1	8.0×10^{-2}

Components found in the skin after 3.5 h of dermal exposure to JP-8 (mean or mean ± SD)

Component	Mass in skin ± SD (mg/g)	C (in JP-8) (mg/ml)	R (x 10^{-3})
undecane	0.266 ± 0.070	48.3	5.5
dodecane	0.143 ± 0.041	36.1	4.0
decane	0.196 ± 0.047	30.2	6.4
tridecane	0.092 ± 0.035	21.9	4.2
nonane	0.077 ± 0.018	9.2	8.4
tetradecane	0.055 ± 0.022	14.6	3.8

R= ratio between mass in the skin and C (in JP-8)

Table 29	Jet fuel
Authors	Riviere *et al*.
Title	Dermal absorption and distribution of topicaly dosed jet fuels Jet-A, JP-8 and JP-8(100)
Source	Toxicology and Applied pharmacology, 160: 60–75, 1999

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* assessment of the percutaneous absorption and cutaneous disposition of topically applied neat Jet-A, JP-8 and JP-8(100)	- aliphatic and aromatic hydrocarbons in different types of jet fuels - isolated perfused porcine skin flap (non-occluded) Analytical method: -liquid scintillation counting	-25 µl of specified jet fuel containing radiolabeled naphthalene and dodecane -duration of exposure: 5 h -exposure area: 5 cm^2 Components measured: -naphthalene in perfusate -dodecane in perfusate -hexadecane in perfusate	Absorption of marker compounds from jet fuels (mean ± SEM, n = 4 for each fuel):

Component	JP-8	Jet-A	JP-8 (Puddle)	JP-8 (100)
^{14}C-Naphthalene				
AUC (%D-h/ml) x 10^{-3}	19.9 ± 2.0	25 ± 2	15 ± 3	26 ± 3
Peak flux (% D/min) x 10^{-3}	15 ± 3	15 ± 1	8 ± 1	16 ± 3
Absorbed (%D)	1.17 ± 0.07	1.49 ± 0.18	1.11 ± 0.16	1.63 ± 0.29
^{3}H-Dodecane				
AUC (%D-h/ml) x 10^{-3}	10.7 ± 0.9	4.8 ± 0.5	3.9 ± 0.4	6.1 ± 0.7
Peak flux (% D/min) x 10^{-3}	3.6 ± 0.4	1.7 ± 0.2	1.4 ± 0.2	2.4 ± 0.1
Absorbed (%D)	0.63 ± 0.04	0.29 ± 0.04	0.27 ± 0.07	0.35 ± 0.04
^{14}C-Hexadecane				
AUC (%D-h/ml) x 10^{-3}	1.7 ± 0.3			
Peak flux (% D/min) x 10^{-3}	1.1 ± 0.2			
Absorbed (%D)	0.18 ± 0.08			

D=dose

It was suggested that the greater absorption of dodecane from JP-8 over Jet-A is caused by one or more additives mixed with Jet-A to make JP-8, which in contrast have minimal influence on naphthalene absorption.

Comment:
Clarification: JP-8 puddle – JP-8 after evaporation after 24 h, resembling JP-composition 24 h after jet fuel spill

Table 30	Jet fuel
Authors	Baynes *et al*.
Title	Mixture effects of JP-8 additives on the dermal disposition of Jet fuel components
Source	Toxicology and Applied pharmacology, 175: 269–281, 2001

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* assessment of the influence if additives (DIEGME, 8Q21 and Stadis450) on dermal disposition of topically applied JP-8	-jet fuel (JP-8) aliphatic and aromatic hydrocarbons - flow through diffusion cells Experiment 1: dermatomated (200-300 µm) perfused porcine skin from the dorsal area of weanling female Yorkshire pigs Experiment 2: isolated perfused porcine skin flaps	Experiment 1: -10 µl of specified jet fuel containing radiolabeled naphthalene (1.21%) and dodecane (4.70%) -duration of exposure: 5 h -exposure area: 0.32 cm^2 Experiment 2: -50 µl of specified jet fuel containing radiolabeled	Absorption of marker compounds from jet fuels in experiment 1 (mean ± SE, n = 4 for each fuel): Component / Max flux x 10^{-4} ($mg/cm^2/h$) / K_p x 10^{-4} (cm/h) / D x 10^{-4} (cm^2/h) ^{14}C-Naphthalene Jet-A: 2.22 ± 0.12 / 1.68 ± 0.06 / 0.95 ± 0.05 Jet-A + DIEGME: 3.18 ± 0.36 / 1.98 ± 0.24 / 1.20 ± 0.13 Jet-A + 8Q21: 2.64 ± 0.12 / 1.68 ± 0.06 / 1.02 ± 0.05 Jet-A + Stadis450: 2.76 ± 0.12 / 1.74 ± 0.06 / 1.04 ± 0.06 Jet-A + DIEGME + 8Q21: 1.24 ± 0.08 / 1.35 ± 0.10 / 0.99 ± 0.03 Jet-A + DIEGME + Stadis450: 1.07 ± 0.14 / 1.13 ± 0.15 / 1.36 ± 0.12 Jet-A + 8Q21 + Stadis450: 0.97 ± 0.03 / 0.97 ± 0.03 / 1.04 ± 0.05 JP-8: 1.86 ± 0.06 / 1.44 ± 0.06 / 0.99 ± 0.05 ^{14}C-Dodecane

<table>
<tr><td>Table 30</td><td colspan="3">Jet fuel</td></tr>
<tr><td>Authors</td><td colspan="3">Baynes et al.</td></tr>
<tr><td>Title</td><td colspan="3">Mixture effects of JP-8 additives on the dermal disposition of Jet fuel components</td></tr>
<tr><td>Source</td><td colspan="3">Toxicology and Applied pharmacology, 175: 269–281, 2001</td></tr>
<tr><td>Type / aim of study</td><td>Test material/ species / technique / detection</td><td>Exposure condition</td><td>Results</td></tr>
<tr><td></td><td>Analytical method:
-liquid scintillation counting</td><td>naphthalene and dodecane
-duration of exposure:
5 h
-exposure area:
5 cm^2

Components measured:
-naphthalene
-dodecane</td><td>
<table>
<tr><td>Jet-A</td><td>0.10 ± 0.01</td><td>0.11 ± 0.01</td><td>1.24 ± 0.29</td></tr>
<tr><td>Jet-A + DIEGME</td><td>0.06 ± 0.003</td><td>0.09 ± 0.01</td><td>1.51 ± 0.10</td></tr>
<tr><td>Jet-A + 8Q21</td><td>0.05 ± 0.01</td><td>0.07 ± 0.01</td><td>4.44 ± 2.33</td></tr>
<tr><td>Jet-A + Stadis450</td><td>0.10 ± 0.01</td><td>0.15 ± 0.02</td><td>3.60 ± 1.57</td></tr>
<tr><td>Jet-A + DIEGME + 8Q21</td><td>0.19 ± 0.02</td><td>0.12 ± 0.01</td><td>19.91 ± 8.48</td></tr>
<tr><td>Jet-A + DIEGME + Stadis450</td><td>0.25 ± 0.05</td><td>0.15 ± 0.03</td><td>5.26 ± 1.83</td></tr>
<tr><td>Jet-A + 8Q21 + Stadis450</td><td>0.30 ± 0.02</td><td>0.18 ± 0.01</td><td>6.55 ± 2.06</td></tr>
<tr><td>JP-8</td><td>0.09 ± 0.01</td><td>0.09 ± 0.01</td><td>1.37 ± 0.14</td></tr>
</table>

Addition of performance additives to Jet-A influenced dodecane absorption and deposition in the stratum corneum to a higher degree than naphthalene absorption and deposition in the stratum corneum.
1.28-5.13% and 0.18-1.78% of dodecane deposition was found in the stratum corneum and dosed skin, respectively. 0.29-0.77% and 0.08-0.47% of deposited naphthalene was found in the stratum corneum and dosed skin, respectively.
Naphthalene peak flux and permeability coefficient were higher than those for dodecane irrespective of Jet fuel or type of membrane model
The data indicated that various combinations of those additives may influence the absorption and disposition of aliphatic and aromatic hydrocarbons present in jetfuel.</td></tr>
</table>

Table 31	Jet fuel		
Authors	Muhammad *et al*.		
Title	Comparative mixture effects of JP-8(100) additives on the dermal absorption and disposition of jet fuel hydrocarbons in different membrane model systems		
Source	Toxicology Letters, 150: 351–365, 2004		
Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* assessment of the influence of additives (MDA, 8Q405 and BHT) on dermal absorption of topically applied JP-8	-jet fuel (JP-8) aliphatic and aromatic hydrocarbons - flow through diffusion cells -silastic membranes (250 µm) -dermatomated porcine skin from the dorsum area (450-550 µm) -isolated perfused porcine skin flaps Analytical method: -liquid scintillation counting	-20 or 50 µl of specified jet fuel containing radiolabelled naphthalene (surface concentration 65 µg/cm^2) and dodecane (surface concentration 175 µg/cm^2) -duration of exposure: 5 h -exposure area: 0.64 and 5 cm^2 Components measured: -naphthalene -dodecane	Absorption parameters following dermal exposure to naphthalene in jet fuel mixtures (mean ± SEM)

Naphthalene	Flux x 10^{-3} (mg/cm^2/h)	K_p ×10^{-3} (cm/h)	Diffusivity ×10^{-3} (cm^2/h)
silastic membrane			
JP-8 (n = 5)	19.29 ± 0.60	1.78 ± 0.06	1.690 ±0.490
JP-8 + MDA (n = 5)	28.23 ± 1.62	2.30 ± 0.13	16.775 ± 5.672
JP-8 + BHT (n = 5)	27.17 ± 0.84	2.22 ± 0.07	3.855 ± 0.907
JP-8 + 8Q405 (n = 4)	26.86 ± 0.71	2.19 ± 0.06	2.164 ± 0.403
JP-8 + MDA + BHT (n = 5)	21.22 ± 0.52	1.99 ± 0.05	1.408 ± 0.411
JP-8 + MDA + 8Q405 (n = 4)	21.65 ± 0.62	2.03 ± 0.06	1.469 ± 0.790
JP-8 + BHT + 8Q405 (n = 4)	21.75 ± 0.73	2.04 ± 0.07	0.251 ± 0.023
JP-8(100) (n = 5)	21.01 ± 0.86	1.93 ± 0.08	6.823 ± 5.836
(pig skin			
JP-8 (n = 5)	2.21 ± 0.27	0.21 ± 0.03	0.455 ± 0.049
JP-8 + MDA (n = 5)	2.63 ± 0.09	0.20 ± 0.01	0.337 ± 0.023
JP-8 + BHT (n = 5)	2.48 ± 0.28	0.19 ± 0.02	0.324 ± 0.039
JP-8 + 8Q405 (n = 4)	2.17 ± 0.16	0.17 ± 0.01	0.330 ± 0.042
JP-8 + MDA + BHT (n = 5)	2.88 ± 0.35	0.23 ± 0.03	0.176 ± 0.015
JP-8 + MDA + 8Q405 (n = 4)	2.64 ± 0.47	0.21 ± 0.04	0.158 ± 0.009
JP-8 + BHT + 8Q405 (n = 4)	2.70 ± 0.48	0.21 ± 0.04	0.161 ± 0.008
JP-8(100) (n = 5)	2.24 ± 0.25	0.21 ± 0.02	0.402 ± 0.037

Comment	Results

Table 31	Jet fuel
Authors	Muhammad *et al*.
Title	Comparative mixture effects of JP-8(100) additives on the dermal absorption and disposition of jet fuel hydrocarbons in different membrane model systems
Source	Toxicology Letters, 150: 351–365, 2004

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results

Naphthalene and dodecane steady state flux, permeability coefficient and diffusivity were higher in silastic membrane than in pig skin regardless of mixture applied.
In both test systems (skin and silasitc membranes) and for both naphthalene and dodecane the values of JP8 and JP8(100) observed for steady state flux, permeability coefficient and diffusivity were similar

Dodecane tissue retention was higher than naphthalene in all membranes studied (data presented only graphically).
On the basis of the results it was concluded that a single membrane system may not be suitable for the final prediction of complex additive interactions in jet fuels on the skin.

Comment:
Clarification: jet fuel JP-8(100) is the same as jet fuel JP-8 and only difference is in added thermal stability performance package to JP-8(100).

Absorption parameters following dermal exposure to dodecane in jet fuel mixtures (mean ± SEM)

Dodecane	Flux x 10^{-3} ($mg/cm^2/h$)	$K_p \times 10^{-3}$ (cm/h)	Diffusivity $\times 10^{-3}$ (cm^2/h)
silastic membrane			
JP-8 (n = 5)	1.46 ± 0.08	0.041 ± 0.002	3.577 ± 1.956 (n=3)
JP-8 + MDA (n = 5)	0.70 ± 0.03	0.020 ± 0.001	9.819 ± 4.607
JP-8 + BHT (n = 5)	0.90 ± 0.07	0.026 ± 0.002	2.187 ± 0.628
JP-8 + 8Q405 (n = 4)	0.75 ± 0.04	0.021 ± 0.001	1.805 ± 0.336
JP-8 + MDA + BHT (n = 5)	0.89 ± 0.15	0.025 ± 0.004	3.741 ± 1.313 (n=4)
JP-8 + MDA + 8Q405 (n = 4)	0.84 ± 0.08	0.024 ± 0.002	3.601 ± 1.822
JP-8 + BHT + 8Q405 (n = 4)	0.74 ± 0.24	0.021 ± 0.007	2.972 ± 1.247
JP-8(100) (n = 5)	1.34 ± 0.05	0.038 ± 0.001	2.484 ± 0.873 (n=3)
(pig skin			
JP-8 (n = 5)	0.090 ± 0.01	0.0025 ± 0.00	1.179 ± 0.331
JP-8 + MDA (n = 5)	0.164 ± 0.03	0.0047 ± 0.00	0.352 ± 0.111
JP-8 + BHT (n = 5)	0.123 ± 0.01	0.0035 ± 0.00	0.455 ± 0.033
JP-8 + 8Q405 (n = 4)	0.171 ± 0.05	0.0049 ± 0.00	0.271 ± 0.047
JP-8 + MDA + BHT (n = 5)	0.077 ± 0.01	0.0022 ± 0.00	2.565 ± 0.778
JP-8 + MDA + 8Q405 (n = 4)	0.097 ± 0.01	0.0028 ± 0.00	1.147 ± 0.330
JP-8 + BHT + 8Q405 (n = 4)	0.079 ± 0.01	0.0022 ± 0.00	1.652 ± 0.435
JP-8(100) (n = 5)	0.094 ± 0.02	0.0027 ± 0.00	1.174 ± 0.384

Table 32	Jet fuel
Authors	Chao *et al.*
Title	Dermal exposure to jet fuel JP-8 significanty contributes to the production of urinary naphtols in fuel-cell maintenance workers
Source	Environmental health perspectives, 114: 182–185, 2006

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vivo* assessment of the contribution of dermal and inhalation exposure to JP-8 to the total body dose	-jet fuel (JP-8) - humans (n = 85) (US Air Force fuel-cell maintenance workers) Analytical method: -GC-MS	Assessment of inhalation exposure: -passive monitors attached to workers shirt collars -duration of exposure: 4 h during work shift Assessment of dermal exposure: -tape striping of three body regions for each worker (three successive tapes per body region) Components measured: -naphthalene in tape strips - 1-naphtols and 2-naphtols in urine	Dermal and inhalation absorption of naphthalene from jet fuels (geometric mean (GM) ± geometric SD (GSD), n = 43-85): Indicator of exposure / GM / GSD / Min / Max x 10^{+4} Dermal naphthalene (ng/m^2) / 4180 / 9.35 / 100 / 509 Breathing-zone naphthalene (ng/m^3) / 614000 / 2.21 / 670 / 391 Preexposure breath naphthalene (ng/m^3) / 492 / 1.99 / 330 / 1.61 Breath naphthalene (ng/m^3) / 9230 / 2.88 / 667 / 7.58 Preexsposure urinary 1-naphthol (ng/l) / 4200 / 3.77 / 242 / 3.90 Urinary 1-naphthol (ng/l) / 28000 / 2.26 / 483 / 12.70 Preexsposure urinary 2-naphthol (ng/l) / 4350 / 3.06 / 424 / 3.79 Urinary 2-naphthol (ng/l) / 38400 / 2.46 / 485 / 31.50 -the contribution of dermal and inhalation exposure, smoking and other covariates to the total body dose of JP-8 were investigated using multiple linear regression analysis -for urinary 1-naphthol breathing-zone naphthalene and smoking were the only significant predictors (explaining 88.2 and 11.8% of total variance, respectively) - for urinary 2-naphthol dermal exposure, breathing-zone naphthalene and smoking were significant predictors (explaining 51.1, 35.8 and 13.1% of total variance, respectively) The results suggested that that dermal exposure to naphthalene contributed significantly to urinary 2-naphthol urinary levels but not to 1-naphthol urinary levels, which is explained as possibly caused by differences in metabolism of naphthalene in the skin by mixed-function oxygenase and conjugating enzymes

Table 33	Jet fuel
Authors	Muhammad *et al*.
Title	Effect of *in vivo* jet fuel exposure on subsequent *in vitro* dermal absorption of individual aromatic and aliphatic hydrocarbon fuel constituents
Source	Journal of Toxicology and Environmental Health, Part A , 68: 719–737, 2005a

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-influence of *in vivo* pre-exposure of skin to jet fuel on *in vitro* percutaneous absorption of selected marker components -the validaty of the use of single-dose application from control skin for the prediction of the dermal penetration after repeated exposures	-jet fuel (JP-8) aliphatic and aromatic hydrocarbons -*In vivo*: female weanling Yorkshire pigs (pre-exposure) -*In vitro*: flow through cells -dermatomed pre-exposed skin (500 µm) Analytical method: -GC-FID	*In vivo*: -two pre-washed cotton fabrics soaked with 2 ml of JP-8 were applied for 1day or for 4 days consecutively by redosing after the first application Two pre-washed cotton fabrics only were applied as controls -duration of exposure: 24 h and 4 times 24 h -exposure area: 12 x 8 cm^2 *In vitro*: -duration of exposure: 24 h -exposure area: 0.64 cm^2 Components measured: -eight aliphatic hydrocarbons -six aromatic hydrocarbons Vehicle for application: Water : ethanol = 50 : 50	Absorption of marker compounds from jet fuel after 1 day of pre-exposure (mean ± SEM, C = control pre-exposure, E = jet fuel pre-exposure, SS = steady-state): Component / SS Flux x 10^{-3} ($mg/cm^2/h$) / Kp x 10^{-3} (cm/h) / Absorption (µg) $Naphthalene_C$ 4.19 ± 0.36 0.16 ± 0.01 10.276 ± 1.023 $Naphthalene_E$ 6.60 ± 0.79 0.26 ± 0.03 17.818 ± 2.255 DMN_C 0.62 ± 0.10 0.02 ± 0.004 1.043 ± 0.202 DMN_E 0.85 ± 0.21 0.03 ± 0.008 1.406 ± 0.447 Ethyl $benzene_C$ 1.04 ± 0.17 0.06 ± 0.009 1.586 ± 0.243 Ethyl $benzene_E$ 3.32 ± 0.52 0.19 ± 0.03 3.137 ± 0.312 TMB_C 1.01 ± 0.14 0.056 ± 0.008 2.177 ± 0.277 TMB_E 1.77 ± 0.21 0.10 ± 0.01 4.192 ± 0.497 CHB_C 0.35 ± 0.06 0.018 ± 0.003 0.548 ± 0.103 CHB_E 0.86 ± 0.11 0.045 ± 0.006 1.651 ± 0.298 o-$xylene_C$ 1.47 ± 0.20 0.085 ± 0.011 2.541 ± 0.335 o-$xylene_E$ 3.80 ± 0.61 0.218 ± 0.04 4.765 ± 0.490 $nonane_C$ 0.03 ± 0.01 0.002 ± 0.001 0.051 ± 0.019 $nonane_E$ 0.08 ± 0.01 0.005 ± 0.001 0.154 ± 0.011 $undecane_C$ 0.07 ± 0.01 0.001 ± 0.000 0.151 ± 0.012 $undecane_E$ 0.16 ± 0.05 0.003 ± 0.000 0.339 ± 0.098 $dodecane_C$ 0.04 ± 0.01 0.0005 ± 0.000 0.081 ± 0.018 $dodecane_E$ 0.06 ± 0.02 0.0009 ± 0.000 0.134 ± 0.037 $tridecane_C$ 0.02 ± 0.01 0.0003 ± 0.000 0.046 ± 0.016 $tridecane_E$ 0.01 ± 0.00 0.0002 ± 0.000 0.034 ± 0.010 TMB-trimethylbenzene, CHB-cyclohexyl benzene, DMN-dimethyl naphthalene

Table 33	Jet fuel
Authors	Muhammad *et al*.
Title	Effect of *in vivo* jet fuel exposure on subsequent *in vitro* dermal absorption of individual aromatic and aliphatic hydrocarbon fuel constituents
Source	Journal of Toxicology and Environmental Health, Part A , 68: 719–737, 2005a

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results

Ethyl benzene, o-xylene and trimethyl benzene showed increase in absorbed amount proportional to the length of exposure. Similarly dodecane showed also increase in absorbed amount, however to a lesser extent.

Steady state flux and permeability coefficient were for all hydrocarbons, (except tridecane in 1-day pre-exposure experiment) higher in the skin pre-exposed to jet fuel than in control in both 1-day and 4-day pre-exposure experiments.
This result suggest that Jp-8 jet fuel pre-exposure of the skin produces changes in skin barrier function and alters the absorption parameters by increasing permeability coefficient and steady state flux as compared to controls.

Absorption of marker compounds from jet fuel after 4 day of pre-exposure (mean ± SEM, C = control pre-exposure, E = jet fuel pre-exposure, SS = steady-state)

Component	SS Flux x 10^{-3} ($mg/cm^2/h$)	Kp x 10^{-3} (cm/h)	Absorption (µg)
$Naphthalene_C$	4.81 ± 0.36	0.19 ± 0.01	12.030 ± 0.989
$Naphthalene_E$	7.57 ± 0.47	0.29 ± 0.02	19.835 ± 1.453
DMN_C	0.67 ± 0.07	0.03 ± 0.003	1.179 ± 0.155
DMN_E	0.99 ± 0.09	0.04 ± 0.004	2.056 ± 0.221
Ethyl $benzene_C$	0.61 ± 0.15	0.035 ± 0.009	0.637 ± 0.136
Ethyl $benzene_E$	2.04 ± 0.17	0.12 ± 0.009	2.594 ± 0.250
TMB_C	0.49 ± 0.04	0.028 ± 0.002	0.837 ± 0.090
TMB_E	1.52 ± 0.10	0.09 ± 0.005	3.821 ± 0.267
CHB_C	0.29 ± 0.02	0.016 ± 0.001	0.470 ± 0.042
CHB_E	0.49 ± 0.07	0.025 ± 0.004	0.852 ± 0.136
o-$xylene_C$	1.02 ± 0.28	0.059 ± 0.016	1.156 ± 0.263
o-$xylene_E$	3.13 ± 0.15	0.180 ± 0.009	4.865 ± 0.340
$nonane_C$	0.03 ± 0.01	0.002 ± 0.000	0.080 ± 0.015
$nonane_E$	0.07 ± 0.01	0.005 ± 0.000	0.178 ± 0.024
$undecane_C$	0.06 ± 0.01	0.001 ± 0.000	0.135 ± 0.022
$undecane_E$	0.10 ± 0.02	0.002 ± 0.000	0.263 ± 0.018
$dodecane_C$	0.02 ± 0.00	0.0002 ± 0.000	0.032 ± 0.007
$dodecane_E$	0.05 ± 0.01	0.0008 ± 0.000	0.112 ± 0.019
$tridecane_C$	0.00 ± 0.00	0.0001 ± 0.000	0.0074 ± 0.0019
$tridecane_E$	0.02 ± 0.01	0.0003 ± 0.000	0.037 ± 0.0093

TMB-trimethylbenzene, CHB-cyclohexyl benzene, DMN-dimethyl naphthalene

Table 34	Jet fuel
Authors	Adami *et al*.
Title	Penetration of benzene, toluene and xylenes contained in gasolines through human abdominal skin *in vitro*
Source	Toxicology In vitro, 20: 1321–1330, 2006

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-evaluation of the amount and the rate of the *in vitro* human skin penetration of volatile aromatic compounds contained in gasolines of different compositions	-gasoline -Franz diffusion cells -Human abdominal full thickness skin (~ 1 mm) Analytical method: -MDGC-FID	- 2 ml of test material (gasoline) was applied on the skin -duration of exposure: 8 h -exposure area: 3.3 cm^2 Components measured: -benzene -xylene -toluene	Absorption parameters for benzene, toluene and xylene from three gasolines (mean ± SD):

Component	Gasoline content (%)	SS Flux x 10^{-3} ($mg/cm^2/h$)	K_p x 10^{-5} (cm/h)	*Total absorption (% of dose)
Gasoline 1				
Benzene	0.74	2.71 ± 1.62	49.19	0.49
Toluene	10.70	5.74 ± 2.77	7.22	0.07
Xylene	14.09	1.01 ± 0.59	0.97	0.01
$aromatics_{tot}$	41.77			
Gasoline 2				
Benzene	0.39	1.80 ± 1.11	63.51	0.63
Toluene	6.05	3.60 ± 2.25	8.20	0.08
Xylene	13.55	0.50 ± 0.25	0.51	0.01
$aromatics_{tot}$	30.15			
Gasoline 3				
Benzene	1.06	1.47 ± 0.53	18.82	0.19
Toluene	6.99	2.07 ± 1.11	4.03	0.04
Xylene	9.26	0.71 ± 0.56	1.04	0.01
$aromatics_{tot}$	29.02			
Gasoline 1,2,3 Mean ± SD				
Benzene		1.99 ± 0.64	43.84 ± 22.82	0.43 ± 0.23
Toluene		3.80 ± 1.84	6.48 ± 2.18	0.06 ± 0.02
Xylene		0.74 ± 0.25	0.84 ± 0.29	0.008 ± 0.003

*total recovery from the receptor fluid, $aromatic_{tot}$ = total aromatic compound present in gasolines

Benzene showed the highest average apparent permeability coefficient and averaged total recovery in receptor fluid as compared to toluene and xylene. The lag times were about 1 h for benzene and about 2 h for toluene and xylene.

The results suggest that increased total aromatic compunds present in various gasolines increase flux of benzene, toluene and xylene and that benzene present major risk for skin permeability compared to toluene and xylene.

Table 35	Jet fuel
Authors	Singh *et al*.
Title	*In vitro* permeability and binding of hydrocarbons in pig ear and human abdominal skin
Source	Drug and Chemical Toxicology, 25: 83– 92, 2002

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-Testing the validity of pig skin as an *in vitro* model for the prediction of percutaneous absorption in humans of some JP-8 chemicals	-jet fuel (JP-8) -Human cadaver abdominal skin dermatomed to 500 µm -Pig ear skin dermatomed to 500 µm -Franz diffusion cells Analytical method: -liquid scintillation counting	-1 ml of of Jp-8 jet fuel containing radiolabeled heptane, hexadecane and xylene which was equal to 2.5 x 10-9 mM/ml JP-8 for heptane and xylene and 1.92 x 10-4 mM/ml JP-8 for hexadecane -duration of exposure: 6 h -exposure area: 1 cm^2 The skin binding behavior of chemicals was determined by mixing of JP-8 jet fuel containing radiolabeled chemicals with the pig or human *stratum corneum* Components measured: -heptane -hexadecane -xylene	Absorption parameters for three chemicals in human and pig skin (mean ± SD): Component / Flux x 10^{-6} ($mg/cm^2/h$) / K_p x 10^{-5} (cm/h) / *Binding Pig skin Heptane: 4.54 ± 0.55 / 18.22 ± 2.20 / 9.22 ± 1.50 Hexadecane: 1.98 ± 0.00 / 4.60 ± 0.00 / 1.23 ± 0.60 Xylene: 2.569 ± 0.312 / 9.68 ± 1.18 / 6.13 ± 1.88 Human skin Heptane: 2.669 ± 0.577 / 10.65 ± 2.31 / 8.83 ± 0.54 Hexadecane: 1.586 ± 0.000 / 3.60 ± 0.00 / 1.53 ± 0.27 Xylene: 2.211 ± 0.021 / 8.33 ± 0.01 / 4.57 ± 0.51 Component / FoD_{perm} / FoD_{bind} Heptane: 1.71 / 1.04 Hexadecane: 1.28 / 0.76 Xylene: 1.16 / 1.31 * binding = Concentration of chemical in 1 g of powdered *stratum corne*um/ Concentration of chemical in 1 g JP-8, FoD_{perm} = permeability through the pig skin/permeability through human skin, FoD_{bind} = binding to pig *stratum corne*um/binding to human *stratum corne*um Heptane showed the highest permeability coefficient (K_p) and binding properties in both pig and human skin compared to hexadecane and xlene. Determined FoD for permeability and binding to the *stratum corne*um between pig and human skin for all chemicals were below factor of two. This suggests that the pig skin could be a good predictor of percutaneous absorption of those chemicals through human skin.

Table 36	PAH
Authors	Yang *et al*.
Title	*In vitro* and *in vivo* percutaneous absorption of benzo[a]pyrene from petroleum crude-fortified soil in the rat
Source	Bulletin of Environmental Contamination Toxicology, 43: 207–214, 1989

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vivo* and *in vitro* assessment of percutaneous absorption of benzo[a]pyrene in petroleum crude oil. Comparison with percutaneous absorption of benzo[a]pyrene sorbed on soil.	- Petroleum crude oil - female Sprague-Dawley rats - dermatomed dorsal skin (thickness 350 µm) Analysis: Mass balance Analytical method: -liquid scintillation spectrometry	Dose: Crude oil spiked with radiolabelled benzo[a]pyrene was administered alone or as a 1% crude-fortified soil in 70-145 µl acetone-carbon disulfide followed by evaporation of vehicle with nitrogen on air. -*In vitro* exposure -exposure duration: 96 h -exposure area: 0.64 cm^2 -*In vivo* exposure -exposure duration: 96 h -exposure area: 7 cm^2 Components measured: -radiolabeled benzo[a]pyrene in excreta, blood and organs	*In vivo* and *in vitro* % dermal absorption of benzo[a]pyrene expressed as % applied dose (mean ± SEM)

Hours after dosing	***In vivo* % of the applied dose	**In vitro* % of the applied dose
Benzo[a]pyrene Crude oil		
24	5.5 ± 1.4	
48	20.1 ± 2.1	
72	27.6 ± 2.1	
96	35.3 ± 2.6	38.0 ± 3.0
Benzo[a]pyrene Crude oil + soil		
24	1.1 ± 0.3	
48	3.7 ± 0.8	
72	5.8 ± 1.0	
96	9.2 ± 1.2	8.5 ± 1.0

*see comment, **urine+feces+tissue

-percentage (%) of the applied dose did not differ significantly between *in vivo* and *in vitro* exposure for both treatments: crude oil and crude oil in soil

-the absorption of benzo[a]pyrene from crude oil alone is 4-5 times higher than from the soil-sorbed crude oil.

-based on *in vitro* and *in vivo* data, it was estimated that the rate of absorption of benzo[a]pyrene from fortified soil is 8.3 x 10^{-9} mg/cm^2/h (0.2 ng/cm^2/day)

Comment:
*The authors did not report actual values for *in vitro* exposure as they did for *in vivo* exposure. Therefore the data in the table were estimated from the graph in the paper

Table 37	PAH
Authors	Dankovic *et al*.
Title	Complex mixture effects on the dermal absorption of benzo[a]pyrene and other polycyclic aromatic hydrocarbons from mouse skin
Source	Journal of Applied Toxicology, 9: 239–244, 1989

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vivo* determination of dermal penetration of benzo[a]pyrene and other PAHs with similar boiling points - evaluation of the effect of co-administration of benzo[a]pyrene with other PAHs on dermal penetration of benzo[a]pyrene -	-radiolabelled benzo[a]pyrene -other high boiling point (800-850 ºF) fractions of PAHs - female CD-1 mice on the dorsal part of the skin Analytical method: -GC-FID -liquid scintillation counting	-Exposure concentration: 50 µl of acetone containing 25 µg of benzo[a]pyrene, or 25 µg of benzo[a]pyrene with 8 mg of the 800-850 ºF PAHs or 25 µg of benzo[a]pyrene with 5 mg of one of the various boiling-point fraction complex organic mixtures (COM) -Exposure duration: not reported -Exposure area: not reported Components measured: -benzo[a]pyrene -benzo[a]pyrene metabolites	The effect of the five complex mixtures on the residence time ($t_{1/2}$) of benzo[a]pyrene on the mouse skin

Component	*BAP $t_{1/2}$ (h)	% of recovered BAP metabolites
BAP	4.3 (3.7 – 5.2)	10.4 (6 h after the end of exposure)
BAP + 300-700 ºF COM	7.8 (6.9 – 9.1)	3.4 (6 h after the end of exposure)
BAP + 700-750 ºF COM	12.9 (10.5 – 16.5)	
BAP + 750-800 ºF COM	14.8 (11.6 – 20.6)	
BAP + 800-850 ºF COM	25.7 (20.3 – 35.1)	
BAP + > 850 ºF COM	29.7 (23.0 – 42.2)	0.2 (24 h after the end of exposure)

BAP = benzo[a]pyrene, COM = complex organic mixtures
*mean and lower and upper 95% confidence interval of the mean

-the $t_{1/2}$ of benzo[a]pyrene increased with increasing boiling point of the added COMs, being almost 7 times greater in the case of > 850 ºF COMs as compared to pure benzo[a]pyrene
-in all cases the addition of COMs prolonged $t_{1/2}$ of benzo[a]pyrene, and therefore reduced the rate of dermal penetration
-the percentage of the benzo[a]pyrene metabolites was reduced by addition of COMs
-it was indicated that the effect of COMs on the dermal penetration of benzo[a]pyrene may be related to the inhibition of the benzo[a]pyrene metabolism at the dermal dosing site
The residence time ($t_{1/2}$) on mouse skin of 12 PAHs contained in a coal-derived COMs were 3 h for benzo[a]pyrene applied alone, 5 h for pyrene, 6.5 h for benzo[a]anthracene, 6.7 h for benzo[a]pyrene, 6.9 h for methylchrysene, 7.3 h for chrysene, 7.4 for 4- or 6-methylchrysene, 7.6 h for C_4-pyrene, 8.1 h for benzo[j or b]fluoranthene, 8.7 h for C2-chrysene and 8.7 h for benzo[e]pyrene.
The determination of the $t_{1/2}$ (ranged from 3 to 8.7 h) of 11 PAHs in the COMs with boiling point of 800-850 ºF suggest that the dermal penetration of benzo[a]pyrene ($t_{1/2}$ = 6.7 h) does appear to be representative of the other PAH components in the mixture and as such might be used as a marker compound of penetration of PAHs

Table 38	PAH
Authors	Turkall *et al*.
Title	A comparative study of the kinetics and bioavailability of pure and soil-adsorbed naphthalene in dermal exposed male rats
Source	Archive of Environmental and Contamination Toxicology, 26: 504–509, 1994

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vivo* determination of pharmaco-kinetics to evaluate potential health risk from occupational or environmental dermal exposure to soil-adsorbed naphthalene	-naphthalene (radiolabelled) - male Sprague-Dawley mice (n = 6) Analysis: Mass balance Analytical method: -liquid scintillation spectrometry -HPLC	-Exposure concentration: 225 µl of ethanol solution containing radiolabelled naphthalene: alone or in combination with 750 mg of soil (surface concentration 3.3 µg naphthalene per cm^2) -Soil type: 1. Atsion sandy soil containing 90% sand, 2% clay and 4.4% organic matter 2. Keyport clay soil containing 50% of sand and 22 % of clay and 1.6% organic mater -Exposure duration: 48 h -Exposure area: 13 cm^2 Components measured: -radiolabelled naphthalene in excreta, blood and organs	Plasma half-lives and AUC plasma concentration time curves of radioactivity following dermal exposure to radiolabeled naphthalene (mean ± SEM) Component / AUC % initial dose/ml h / $t_{1/2}$ / h absorption / $t_{1/2}$ / h elimination Naphthalene alone: 0.50± 0.04 / 2.1 / 12.8 Naphthalene + sandy soil: 0.42 ± 0.03 / 4.6 / 20.0 Naphthalene + clay soil: 0.63 ± 0.03 / 2.8 / 15.3 The three main metabolites found in the urine were 2,7-dihydroxynaphthalene, 1,2-dihydroxynaphthalene and 1,2-naphthoquinone, while to the lesser extent also naphthalene,1-naphthol and 2-naphthol were observed in rats. The unidentified metabolites of naphthalene present in urine comprise 25% of total radioactivity. No significant differences were observed between excreted amounts of metabolites for different treatments. The naphthalene derived-radioactivity was rapidly excreted in urine after the pure and clay soil treatment. Between 70 and 87 % of radioactivity was recovered in urine, while 6 to 14 % was found in expired air. Sandy soil treated naphthalene showed a slower excretion of radioactivity, although the recovery in urine was similar to the other two treatments. Only 0.9% was found in expired air. The data indicated a larger affinity of sandy soil for naphthalene than for the clay. This is supported by slower urinary excretion, a significant decrease in expired air and a shifted secondary excretion from expired air to faeces. Additionally, the washes of the application site contained significantly higher radioactivity when treated with sandy soil compared to naphthalene alone treatment.

Table 39	PAH
Authors	Van Rooij *et al*.
Title	Absorption of polycyclic aromatic hydrocarbons through human skin: differences between anatomical sites and individuals
Source	Journal of Toxicology and Environmental Health, 38: 355–368, 1993

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vivo* determination of dermal uptake of PAHs in humans Contribution of various anatomical sites to uptake. Assessment of interindividual variation	-therapeutical coal-tar ointment containing 10% coal-tar in a vehicle of zink oxide paste - male volunteers (n = 9) Analytical method: -HPLC -luminescence	Experiment 1 Exposure surface concentration 2.5 mg/cm^2 of coal-tar -Exposure duration: 45 min -Exposure area: 24 cm^2 Experiment 2 Exposure surface concentration 2.5 mg/cm^2 of coal-tar -Exposure duration: 6 h -Exposure area: 400 cm^2 -Exposure sites: forehead, shoulder, volar forearm, palm site of hands, groin and ankle Components measured: -disappearance of PAHs from the surface of the skin (experiment 1) -level of PAH metabolite 1-OH-pyrene in urine (experiment 2)	Skin absorption rate constants of PAHs (mean, n = 4)and excreted amount of 1-OH-pyrene in urine (mean, n = 4) at different anatomical sites **Site** / **Absorption rate constant (1/h) (from experiment 1)** / **Site** / **Excreted amount of 1-OH-pyrene (nmol) (from experiment 2)** Shoulder 0.135 (0.069 – 0.196) — Neck 14.6 (10.1 – 23.8) Forearm 0.070 (0.060 – 0.089) — Calf 13.9 (7.0 – 23.0) Forehead 0.065 (0.046 – 0.083) — Forearm 11.3 (5.0 – 21.2) Groin 0.053 (0.038 – 0.083) — Trunk 10.8 (7.8 – 15.0) Hand (palm) 0.037 (0.026 – 0.050) — Hand 7.7 (6.0 – 11.1) Ankle 0.036 (0.028 – 0.040) Mean ± SD 0.066 ± 0.037 — Mean ± SD 11.6 ± 2.8 -The PAH absorption rate constants ranged from 0.026/h to 0.196/h. -Two-way ANOVA showed significant effect of the anatomical site on the absorption rate constant but no significant effect of the individuals on the absorption rate constants. The variance explained 67% by skin site and only 7% by interindividual differences -The total excreted amount of 1-OH-pyrene ranged from 5.0 to 23.8 nmol -two-way ANOVA showed that there are significant differences in the total excreted amount of 1-OH-pyrene between individuals, but no significant differences in the extent of urinary 1-OH-pyrene excretion between various skin sites -there was a significant difference between individuals for time needed in which half of the 1-OH-pyrene was excreted and it varied 8.2 to 18.9 h (data were not shown) -based on the absorption rate constants determined in the surface disappearance experiments, the authors estimated that at a low PAH (more comparable to occupational situations) dose 20-56% will be absorbed after 6 h.

Table 40	PAH		
Authors	Van Rooij *et al*.		
Title	Dermal absorption of polycyclic aromatic hydrocarbons in the blood-perfused pig ear		
Source	Journal of Applied Toxicology, 15: 193–2009, 1995		
Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* assessment of the validity of pyrene as a representative marker compound for the dermal absorption of other PAHs	-industrial coal-tar - isolated blood perfused pig ear skin Analytical method: -HPLC	Exposure surface concentration 11 mg/cm^2 of coal-tar -Exposure duration: ≤ 250 min -Exposure area: 24 cm^2 Components measured: -11 PAHs (see table results)	The content of PAHs in coal tar, amount absorbed and their flux through pig ear skin during 200 min after coal tar application (means, or means ± SD)

Site	Content in coal tar (%)	#Relative amount absorbed x 10^{-6} (mg/cm^2)	*Flux x 10^{-4} (mg/cm^2/h)
Fluorene	2.1	1.58 ± 0.65	0.71
Phenanthrene	6.8	2.14 ± 0.68	1.04
Anthracene	3.7	0.32 ± 0.09	0.20
Fluoranthene	4.0	0.29 ± 0.14	0.21
Pyrene	2.1	0.20 ± --	0.12
Benzo[b]fluoranthene	0.9	< 0.03 ± 0.03	0.007
Benzo[k]fluoranthene	0.4	< 0.005 ± 0.005	< 0.003
Benzo[a]pyrene	0.9	< 0.033 ± 0.051	0.008
Indenol[123-cd]pyrene	0.6	< 0.003 ± 0.006	< 0.003
Dibenzo[ah]anthracene	0.4	< 0.008 ± 0.008	< 0.003

*At 200 min after coal tar application, the exact data were not shown in the article
#Related to pyrene

-the use of pyrene as a marker of PAH absorption through pig skin will underestimate the cumulative absorption of PAHs with lower molecular weight, and overestimate the absorption of those with higher molecular mass.
-it was suggested that this situation is likely to occur in human skin as well

Table 41	PAH
Authors	Roy *et al.*
Title	Studies estimating the dermal bioavailability of polynuclear aromatic hydrocarbons from manufactured gas plant tar-contaminated soils
Source	Environmental Science and Technology, 32: 3113–3117, 1998

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* determination of the dermal penetration of PAHs in manufactured gas plant (MGP) tar-contaminated soil. comparison with the dermal penetration of the same PAHs in soil extracts.	-PAHs in MGP contaminated soil -PAHs in soil extracts - dermatomed male and female human cadaver abdominal skin (350 µm) Static diffusion cells Analysis: Mass balance Analytical method: -liquid scintillation counting -GC-MS	-Exposure concentration: Soil or soil extracts samples were spiked with radiolabelled benzo[a]pyrene. The samples already contained a group of targeted PAHs at levels ranging from 10 to 2400mg/kg and from 12000 to 34000 mg/kg, respectively. -Exposure duration: 120 h -Exposure area: 1.8 cm^2 Components measured: -radiolabelled benzo[a]pyrene representing target PAHs	Percentage of applied dose in receptor fluid and skin, flux and dermally absorbed dose (DAD) values of benzo[a]pyrene as surrogate for the target PAHs in MGP contaminated soil and soil extracts (mean, or mean ± RSD)

*Sample-target PAH (mg/kg)	% applied dose in receptor fluid	% applied dose in the skin	Flux x 10^{-6} ($mg/cm^2/h$)	Dermally absorbed dose (mg/kg/day)
A150L/14	0.69 ± 0.10	0.6 ± 0.1	0.025 ± 0.21	2.3×10^{-6}
B150L/10	0.19 ± 0.10	1.0 ± 0.4	0.0064 ± 0.22	6.0×10^{-7}
C150L/38	1.00 ± 0.40	0.9 ± 0.3	0.29 ± 0.43	2.7×10^{-5}
A150M/140	0.57 ± 0.20	0.5 ± 0.1	0.19 ± 0.26	1.8×10^{-5}
B150M/52	0.46 ± 0.20	0. 9 ± 0.3	0.059 ± 0.37	5.5×10^{-6}
C150M/170	0.53 ± 0.10	0.4 ± 0.1	0.48 ± 0.23	4.5×10^{-5}
A150H/1500	0.30 ± 0.10	0.6 ± 0.3	1.00 ± 0.35	9.4×10^{-5}
B150H/870	0.49 ± 0.30	0. 9 ± 0.4	0.83 ± 0.51	7.8×10^{-5}
C150H/2400	0.20 ± 0.10	0.7 ± 0.3	2.20 ± 0.53	2.0×10^{-4}
A150H-EXT/12000	1.36 ± 0.20	0.7 ± 0.3	210 ± 0.19	2.0×10^{-2}
B150H-EXT/34000	2.37 ± 0.40	1.8 ± 0.8	750 ± 0.520	7.1×10^{-2}
C150H-EXT/32000	6.50 ± 2.60	2.5 ± 0.7	360 ± 0.34	3.4×10^{-2}

*A/B/C indicate MGP site, L/M/H/EXT indicate low, medium, high soil concentration and soil extract, 150 = < 150 µm particle size

Results from three MGP contaminated sites showed reduction of 2-3 orders of magnitude in PAH absorption through human skin from the most contaminated soils as compared to soil extracts.
This reduction in PAH penetration can be attributed to PAH concentration and soil properties (content of organic matter), i.e. binding effects according to the authors.

Table 42	PAH
Authors	Sartorelli *et al.*
Title	Dermal exposure assessment of polycyclic aromatic hydrocarbons: *in vitro* percutaneous penetration from lubricating oil
Source	International Archive of Occupational and Environmental Health, 72: 528–532, 1999

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* percutaneous penetration of PAHs. Effect of the application form /vehicle	-mixture of PAHs - static diffusion cells -full thickness monkey skin Analysis: Mass balance Analytical method: -HPLC	-Exposure surface concentration: PAHs were applied in lubrication oil or in acetone (ranging from 6.1 to 160 nmol/cm^2) -Exposure duration: Not clearly specified -Exposure area: - 1.77 cm^2 Components measured: -13 PAHs (see table results)	Steady-state permeability coefficient (Kp), fluxes and lag time of the tested PAHs applied in lubrication oil or acetone(mean ± SD)

Component	$K_p \times 10^{-3}$ (cm/h) lubricating oil	$K_p \times 10^{-3}$ (cm/h) acetone	t_{lag} (h) lubricating oil	t_{lag} (h) Acetone
Naphthalene	1.87 ± 1.31	6.31± 2.49	4.86 ± 7.99	1.18 ± 0.01
Acenaphthalene	1.72 ± 1.76	7.80 ± 4.10	8.37 ± 3.44	2.34 ± 2.31
Fluorene	1.64 ± 1.66	6.56 ± 5.33	5.70 ± 3.02	4.23 ± 3.99
Anthracene	0.93 ± 0.98	3.97 ± 2.82	17.55 ± 4.73	12.85 ± 7.18
Phenanthrene	0.50 ± 0.28	2.63 ± 0.74	15.15 ± 3.10	10.95 ± 7.62
Pyrene	0.17 ± 0.04	4.13 ± 4.36	13.38 ± 8.91	24.46± 2.68
Benzo[a]anthracene	*	1.72 ± 2.60	*	27.14 ± 8.28
Chrysene	0.22 ± 0.12	0.57 ± 0.43	26.12 ± 3.34	23.79 ± 2.25
Benzo[b]fluoranthene	*	0.09 ± 0.04	*	22.46 ± 21.12
Benzo[k]fluoranthene	*	0.09 ± 0.04	*	23.80 ± 25.70
Benzo[a]pyrene	*	0.23 ± 0.20	*	31.21 ± 10.81
Dibenzo[ah]anthracene	*	*	*	*
Benzo[ghi]perilene	*	*	*	*

*below detection limit

The permeability results showed that the transport of PAHs through the skin is slower from lubricating oil than from acetone.This could be attributed to the liposolubility of PAHs and their affinity for oily liquids.
The permeability was significantly lower for naphthalene, acenaphthalene, fluorene, anthracene, phenanthrene and pyrene when applied in lubricating oil, except for chrysene where no significant difference was observed.
This was attributed to the fact that very often the concentration of chrysene was below the detection limit.

Comment:
No explicit information on exposure duration

Table 43	PAH		
Authors	Potter *et al*.		
Title	Studies on the dermal and systemic bioavailability of polycyclic aromatic compounds in high viscosity oil products		
Source	Archive of Toxicology, 73: 129–140, 1999		
Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vivo* and *in vitro* assessment of the bioavailability of benzo[a]pyrene in base oil, residual aromatic extracts and bitumens determination of influence of viscosity on the bioavailability	- base oils -residual aromatic extracts -bitumens *in vivo* -female CF1 mice (dorsal skin) *in vitro* - full thickness human skin Analytical method: -liquid scintillation spectrometry -HPLC	Experiments 1 (mice): 80 µl of oil solution or residual aromatic extracts containing 0.1% of radiolabelled benzo[a]pyrene Experiment 2 (mice): 50 µl of oil solution or bitumen discs containing 0.1% of radiolabeled benzo[a]pyrene Human skin experiment: Skin was exposed to 0.1% of radiolabeled benzo[a]pyrene in oil, residual aromatic extracts or bitumens -Exposure duration: 6 h (mice and human) -Exposure area: 10 cm^2 (mice) 4.91 cm^2 (human skin) Components measured: -radiolabeled benzo[a]pyrene in blood -benzo[a]pyrene DNA adducts	Viscosity of different oils, RAE-s and bitumens used as a vehicle in the experiments Component / Viscosity (cSt) Oil A: 32 Oil B: 198 *RAE C: 5400 RAE D: 4800 RAE E: 5160 Bitumen 1: 0.65×10^6 Bitumen 2: 3.60×10^6 Bitumen 3: 31×10^6 Bitumen 4: 69×10^6 *RAE = residual aromatic extracts Results in mice have shown significant reduction in the binding of radioactivity from ^{14}C benzo[a]pyrene to both DNA and blood as the viscosity of the oil product increased in the range from 32 to 5000 cSt. Apparently, there was linear relationship between decrease in binding with increase in the logarithm of viscosity The bioavailability of the ^{14}C benzo[a]pyrene was significantly reduced with increase of the viscosity of the vehicle. This trend was continued with bitumens which have even higher viscosity, binding of radioactivity from radiolabelled benzo[a]pyrene to DNA and blood decreased with increased viscosity, but to a lesser extent Experiments with human skin *in vitro* gave similar results of decreased binding of radiolabelled benzo[a]pyrene to DNA as viscosity of the vehicle increased, however there was no significant difference between RAE and bitumen vehicle Comment: Results were shown graphically without explicit values

Table 44	PAH
Authors	Sartorelli *et al.*
Title	Dermal exposure assessment of polycyclic aromatic hydrocarbons: *in vitro* percutaneous penetration from coal dust
Source	Toxicology and industrial Health, 17: 17–21, 2001

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* assessment of the dermal penetration of PAHs from coal dust through human skin comparison with the dermal penetration of pure PAH components	-coal dust - dermatomed cadaver human abdominal skin (thickness 350 µm) -static diffusion cells Analytical method: -HPLC	Exposure surface concentration: -9 mg/cm^2 of coal dust applied by adding a minimal amount of acetone for correct distribution on the skin surface -a mixture of seven PAHs was applied in 30 µl of acetone without occlusion -Exposure duration: 72 h -Exposure area: 1.77 cm^2 Components measured: -PAHs (see table results)	Percentages of absorption of PAHs applied in acetone solution at (mean ± SD)

	Percentage at			
Component	6 h	24 h	48 h	72 h
Phenanthrene	9.58± 4.70	32.66± 14.41	39.26± 19.83	43.54±
Pyrene	1.86± 2.05	15.18± 7.01	37.51± 8.06	25.08
Benzo[a]anthracene	0.42± 0.28	4.54± 1.78	9.88± 3.50	51.98±
Benzo[b]fluoranthene	*	1.10± 0.79	3.71± 1.74	14.97
Benzo[k] fluoranthene	*	0.97± 0.57	3.45± 1.54	14.22± 5.06
Benzo[a]pyrene	*	1.40± 0.78	4.95± 2.05	6.75± 3.46
Dibenzo[a,h]anthracene	*	*	0.60± 0.38	6.19± 2.88
				8.57± 3.67
				1.94± 1.34

*below detection limit

Since the concentration of PAHs in the receptor fluid after exposure to coal dust was always under the detection limit, no percutaneous penetration of PAHs could be assessed.
It was suggested that the absence of detectable penetration of PAHs from coal dust could be attributed to the physico-chemical properties of coal

Pure PAH compounds in acetone
Data on cumulative percutaneous penetration showed slower penetration of dibenzo[a,h]anthracene as compared to other PAHs, while the percutaneous penetration of phenanthrene was faster, decreasing after 24 h.

Table 45	PAH
Authors	Stroo *et al.*
Title	Dermal bioavailability of benzo[a]pyrene on lampblack: implications for risk assessment
Source	Environmental Toxicology and Chemistry, 24: 1568–1572, 2005

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* assessment of percutaneous penetration of benzo[a]pyrene from lampblacks Evaluation of different lampblacks and lampblack/soil mixtures from manufactures gas plants (MGP)	- lampblacks - lampblack/soil mixtures - human cadaver split thickness skin (thickness 350 µm) Static diffusion cells Analytical method: -HPLC	-Exposure surface concentration: 10 mg/cm^2 -Exposure duration: 96 h -Exposure area: 1.77 cm^2 Components measured: -benzo[a]pyrene	Benzo[a]pyrene concentration in applied lampblack samples and dermal flux (mean or mean ± 95% confidence interval)

Sample	Concentration in samples (mg/kg)	Steady-state flux x 10^{-6} (mg/cm^2/h)	*Adjusted flux x 10^{-9} (mg/cm^2/h)	Total carbon content (%)
CA-2	915	0.200± 0.080	13	76.9
CA-5	135	0.100± 0.030	6.7	12.9
CA-10	1702	0.300± 0.080	20	82.9
CA-13	111	0.050± 0.060	3.3	6.2
CA-14	38	0.050± 0.080	3.3	6.2
CA-17	817	0.300± 0.080	20	62.4
CA-18	632	0.200± 0.080	13	24.2

*measured benzo[a]pyrene flux was adjusted by multiplying by the assumed soil adherence factor of 0.2 mg/cm^2 (default factor according to Dermal exposure assessment by US EPERCUTANEOUS ABSORPTION) and then dividing by the soil monolayer coverage value of 3 mg/cm^2 (default factor used in the method for calculation of risk assessment by Californian EPERCUTANEOUS ABSORPTION).

In comparison to freshly added PAHs to the soil matrix, dermal fluxes of aged native PAHs measured in this study, were low and the these results suggests tighter binding of aged native PAHs to the lampblack.

The experimentally determined fluxes correlated well with applied soil-PAH concentrations and therefore suggest a strong influence of the concentration of PAHs on measured fluxes (r^2 = 0.86)
Similar results were obtained for the correlation of measured fluxes with total carbon (highly aromatic carbon matrix) content of the soil (r^2 = 0.87).

Table 46	Nonane, dodecane, tetradecane		
Authors	Babu *et al*.		
Title	Percutaneous absorption and skin irritation upon low-level prolonged dermal exposure to nonane, dodecane and tetradecane in hairless rats		
Source	Toxicology and Industrial Health, 20: 109–118, 2004		
Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* assessment of the percutaneous absorption of aliphatic hydrocarbons evaluation of impact of prolonged dermal exposures to aliphatic hydrocarbons of varied chain length on skin irritation and biomarker responses. Evaluation ofthe relation to the skin permeation and retention of these chemicals	-nonane -decane -tetradecane - dorsal skin from male CD hairless rats Static diffusion cells Analysis: Mass balance Analytical method: -liquid scintillation spectrometry -HPLC	-Exposure concentration: 0.5 ml of mixture of aliphatic hydrocarbons containing their radiolabelled counterparts -Exposure duration: 8 h -Exposure area: 0.636 cm^2 Components measured: -radiolabelled nonane, decane, tetradecane	The data showed that the steady-state flux of dodecane was about 3 folds higher than for nonane and about 77 fold higher than for tetradecane For all three aliphatic hydrocarbons the measured lag time was about 1 h. The retention of chemicals was much higher in the *stratum corneum* than in the epidermis and dermis at all measured time points The retention in the epidermis and dermis was highest for dodecane followed by nonane and tetradecane. The relationship between molecular weight of the aliphatic hydrocarbons and skin retention in epidermis and dermis showed to be parabolic, which is in contrast to the *stratum corneum* where the relationship between molecular weight of aliphatic hydrocarbons (carbon chain length) and the absorption in the *stratum corneum* was linear. Comment: The data were presented only graphically

Table 47	Jet fuel
Authors	Kanikkannan *et al*.
Title	Percutaneous permeation and skin irritation of JP-8 + 100 jet fuel in a porcine model
Source	Toxicology Letters,119: 133 - 142, 2001b

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* assessment of percutaneous permeation of JP-8 + 100 In vitro assessment of the effect of three performance additives (BHT, MDA and 8Q405 on percutaneous permeation of JP-8 across pig ear skin	Test material: - JP-8 - JP-8 + 100 - JP-8 + BHT - JP-8 + MDA - JP-8 + 8Q405 Experimental method and species: - dermatomed pig ear skin (500 µm) Static (Franz) diffusion cells Analytical method: -liquid scintillating counting	-1 ml of jet fuels as described in column two spiked with radiolabelled tridecane, nonane, naphthlane and toluene -duration of exposure: 24 h -exposure area: 1.1 cm^2 Components measured: -tridecane -nonane -naphthlane -toluene	Steady state (SS) flux (mean ± SD) of tridecane, nonane, naphthalene and toluene from JP-8, JP-8 + 100 and JP-8 + additives across pig ear skin

SS Flux x 10^{-3} ($mg/cm^2/h$)	Tridecane (% (w/w) in jet fuel = 2.7 %)	Nonane (% (w/w) in jet fuel = 1.1 %)	Naphthalene (% (w/w) in jet fuel = 0.26 %)	Toluene (% (w/w) in jet fuel = 0.06 %)
JP-8*	1.508 ± 0.188	0.477 ± 0.025	0.376 ± 0.017	0.119 ± 0.004
JP-8 + 100	1.318 ± 0.155**	0.395 ± 0.007**	0.419 ± 0.033**	0.094 ± 0.001**
JP-8 + BHT	1.223 ± 0.059**	0.396 ± 0.014**	0.327 ± 0.015**	0.071 ± 0.013**
JP-8 + MDA	1.530 ± 0.111	0.451 ± 0.031	0.386 ± 0.020	0.114 ± 0.009
JP-8 + 8Q405	1.465 ± 0.093	0.461 ± 0.033	0.364 ± 0.037	0.117 ± 0.005

K_p x 10^{-4} (cm/h)				
JP-8 + 100	0.6102	0.4489	2.014	1.958

*Data reproduced from Kanikkannan et al., 2001a (Table 25), **significantly different ($p<0.05$) compared to JP-8.

The permeation of tridecane was highest followed by nonane, naphthalene and toluene for all jet fuels.
The steady-state flux of all components showed to be significantly lower from JP-8 + 100 and JP-8 + BHT as compared to chemicals from JP-8, except the steady-state flux of naphthalene from JP-8 + 100 which was significantly higher than from JP-8. It was suggested that BHT minimizes the changes induced in the skin by inhibiting the formation of oxidative products and free radicals from JP-8.
MDA and 8Q405 showed no significant effect on the permeation of chemicals from JP-8.

Comment:
BHT – butylated hydroxytoluene (antioxidant), MDA – metal deactivator,
8Q405 – detergent/dispersant
JP-8 +100 - (contains all three additives BHT, MDA and 8Q405)

Table 48	Toluene, m-xylene
Authors	Kezic *et al*.
Title	Dermal absorption of neat liquid solvents on brief exposures in volunteers
Source	American Industrial Hygiene Association journal, 62: 12 - 18, 2001

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vivo* investigation of dermal absorption of neat liquids, toluene and m-xylene, after brief exposure	Test material: - neat toluene - neat m-xylene -human volunteers (n = 6) Data analysis: -by linear system dynamics (LSD, Opdam, 1991) using a reference exposure via inhalation and (de)convolution to yield dermal permeation rate time courses Analytical method: -GC-FID	- Inhalation (reference) exposure: -exposure concentration was below the occupational. exposure limit (188 and 434 mg/m^3 for toluene and xylene, respectively) -exposure duration: 10 min Dermal exposure: - a bottomless glass chamber affixed onto volar forearm at two-thirds of the distance from wrist to the elbow -duration of exposure: 3 min -exposure area: 27 cm^2 Components measured: -xylene and toluene in exhaled air as biomarkers of exposure	Average dermal flux into the skin of neat chemicals and estimated dermal uptake Chemical | Flux x 10^{-3} (mg/cm^2/h) | *Dermal uptake (mg) Toluene | 1.23 ± 0.44 | 175 m-xylene | 0.29 ± 0.11 | 43 Comment: Extrapolated linearly to the skin area of 360 cm^2 and exposure duration of 3 minutes repeated eight times during a working day

Table 49	Benzene
Authors	Wester and Maibach
Title	Benzene percutaneous absorption: Dermal exposure relative to other benzene sources
Source	International Journal of Occupational and environmental Health, 6: 122 - 126, 2000

Type / aim of study	Test material/ species / technique / detection	Exposure condition	Results
-*In vitro* investigation of dermal absorption of benzene as water or toluene solution at different concentration level Comparison of dermal absorption relative to the other sources of exposure	Test material: - benzene in toluene - benzene in water -human skin (n = 4) Technique: -not reported	-benzene in toluene as 0.15, 0.1, 0.05 and 0.01 % solution -benzene in water as 0.1 and 0.05 % solution Exposure surface concentration: 5 µl/cm^2 -duration of exposure: not reported -exposure area: not reported Components measured: -benzene	*In vitro* percutaneous absorption of benzene expressed as %Dose absorbed (mean ± SD)

Chemical	% Dose absorbed					
Chemical	C (%)	Receptor fluid	Epidermis	Dermis	Wash	Total
Benzene in toluene	0.15	0.10 ± 0.08	0.03 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.17 ± 0.07
	0.10	0.11 ± 0.08	0.04 ± 0.02	0.01 ± 0.01	0.04 ± 0.02	0.19 ± 0.07
	0.50	0.08 ± 0.03	0.04 ± 0.02	0.01 ± 0.01	0.05 ± 0.01	0.17 ± 0.03
	0.01	0.12 ± 0.06	0.03 ± 0.01	0.01 ± 0.00	0.04 ± 0.01	0.19 ± 0.06
Benzene in water	0.10	5.03 ± 1.88	0.42 ± 0.33	0.27 ± 0.16	1.82 ± 0.86	7.53 ± 1.04
	0.50	3.88 ± 1.71	0.36 ± 0.51	0.41 ± 0.65	1.50 ± 1.45	6.16 ± 4.13

Benzene showed no dose response in terms of % benzene absorbed (receptor fluid + skin content) when applied in toluene.
The small total % of the dose absorbed (0.17 - 0.19 %) indicated that most of the benzene evaporated along with the toluene.

Benzene applied in water showed greater absorption (approximately 29- to 44-fold increase in absorption for receptor fluid + skin content) compared to benzene applied in toluene.
This could be explained by less volatility of water, which retained solubilized benzene on the skin longer, compared to toluene.

Absorption of benzene through the skin exposure seems to be of minor importance (negligible) in comparison with estimates of daily ingestion (estimated as much as 250 µg/day from foods) and from other exposures such as smoking (1800ug), passive smoking (50 µg), filing a gas tank (10 µg), driving a car (40µg) or just breathing outdoor air (120 µg).

Comment: the author did not reported experimental conditions; exposure area, duration of exposure, thickness of the skin and experimental technique (static or flow through diffusion cells) nor the analytical technique

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