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A NEW TECHNIQUE TO ASSESS DERMAL ABSORPTION OF CHEMICAL VAPOR IN VITRO BY THERMOGRAVIMETRIC ANALYSIS (TGA)

Matias Rauma

Stockholm 2008
"In the country of the blind, the one-eyed person is king"

Jean-Jacques Rousseau
1712-1778

To My Family
ABSTRACT

There is a huge lack of dermal uptake data for chemicals, and it is frequent with large variations in reported permeability coefficients for chemicals with more than one data set, showing the need for a new and standardized in vitro method. The overall aim of this thesis was to develop the new method of measuring dermal absorption of chemical vapor using the TGA method.

Assessment of dermal absorption by TGA (Study I)
Round pieces (ø8 mm) of pig epidermis were placed on a platinum pan and inserted into the TGA furnace, where a constant temperature (35 ± 0.01°C) and humidity (45% RH) were maintained. The weight change of the skin was readily recorded prior, during and after exposure to an airflow containing known concentrations of chemical vapor (from water, 2-propanol, methanol and toluene). When no chemical vapor was present the weight of the skin was constant and upon addition of chemical vapor the weight of the skin increased until an equilibrium with the surrounding air was reached. After removal of chemical vapor the skin weight decreased until the baseline weight was reached. The shape of the weight curves was unique for each studied chemical, were the slope and total weight increase reflect the diffusion and skin:air partition coefficients of the studied chemicals, respectively.

Vapor generating system (Study II)
A computer-controlled, fully automated vapor generating system was created. The chemical vapor was generated by means of bubbling clean, dry air through tailor-made stainless steel chemical containing gas wash bottles, placed in a thermo regulated water bath. Magnetic valves were placed before and after each gas wash bottle to direct the airflow and to prevent back flow, respectively. The performance of a gas wash bottle was tested and close to full saturation was measured using cyclohexanone as test substance. A dedicated computer program was created allowing automated execution of schedules, facilitating precise timing and long time dermal uptake studies using TGA.

Development of TGA-specific diffusion model (Study III)
A multi compartmental, parallel diffusion model was created using a derived TGA-specific solution to Fick’s second law of diffusion. Diffusion and skin:air partition coefficients were obtained by fitting the model to TGA obtained skin weight curves from studies on n-butyl acetate, methanol, 2-propanol and toluene. Akaike Information Criterion (AIC) was applied and it was found that a two compartment model was sufficient to describe the weight curves. The first compartment was fast and having a low capacity whereas the second compartment was slower with a higher capacity.

Validate TGA method against Franz cells (Study IV)
TGA and Franz diffusion cell studies were preformed on pig skin testing dermal penetration of the above mentioned chemicals and n-butanol, cyclohexanone, ethyl benzene, n-hexane, styrene and m-xylene. The Franz cell obtained diffusion coefficients showed good agreement with the faster of the two TGA compartments.

In conclusion, skin weight changes were readily recorded when dermal exposure to chemical vapor was studied using the TGA method. Diffusion and partition coefficients were obtained by fitting a TGA-specific two compartment model to the recorded weight curves. A good agreement was found when comparing the TGA obtained diffusion coefficients with those from Franz cell experiments.
LIST OF PUBLICATIONS

I. **Matias Rauma**, Tina S Isaksson and Gunnar Johanson
   *A new technique to assess dermal absorption of volatile chemicals in vitro by thermal gravimetric analysis.*
   Toxicology In Vitro, 2006, 20:1183-1189
   *2006 Paper of the Year Award
   Dermal Toxicology Specialty Section, Society of Toxicology*

II. **Matias Rauma** and Gunnar Johanson
    *A computer-controlled system for generation of chemical vapours in in vitro dermal uptake studies.*
    Skin Research and Technology, 2007, 13:79-83

III. **Matias Rauma** and Gunnar Johanson
     *Assessment of dermal absorption by thermogravimetric analysis – development of a diffusion model based on Fick’s 2nd law*
     Submitted

IV. **Matias Rauma** and Gunnar Johanson
    *Comparison of the thermogravimetric analysis (TGA) and Franz cell methods to assess dermal diffusion of volatile chemicals*
    Submitted
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<table>
<thead>
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<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>A</td>
<td>Activity</td>
</tr>
<tr>
<td>A</td>
<td>Area – (cm²)</td>
</tr>
<tr>
<td>AIC</td>
<td>Akaike's information criterion</td>
</tr>
<tr>
<td>C</td>
<td>Concentration – (mg/cm³)</td>
</tr>
<tr>
<td>C&lt;sub&gt;donor&lt;/sub&gt;</td>
<td>Concentration of the chemical in donor of diffusion cell – (mg/cm³)</td>
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<tr>
<td>C&lt;sub&gt;receptor&lt;/sub&gt;</td>
<td>Concentration of the chemical in receptor of diffusion cell – (mg/cm³)</td>
</tr>
<tr>
<td>C&lt;sub&gt;TGA&lt;/sub&gt;</td>
<td>Concentration of the chemical in TGA furnace – (mg/cm³)</td>
</tr>
<tr>
<td>D</td>
<td>Diffusion coefficient, or diffusivity – (cm²/s)</td>
</tr>
<tr>
<td>D&lt;sub&gt;F&lt;/sub&gt;</td>
<td>Diffusion coefficient from Franz diffusion cell study – (cm²/s)</td>
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<tr>
<td>D&lt;sub&gt;x&lt;/sub&gt;</td>
<td>Diffusion coefficient of compartment x – (cm²/s)</td>
</tr>
<tr>
<td>h</td>
<td>Skin thickness – (cm)</td>
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<tr>
<td>J</td>
<td>Flux – (g/cm²/h)</td>
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<tr>
<td>J&lt;sub&gt;SS&lt;/sub&gt;</td>
<td>Steady-state flux – (g/cm²/h)</td>
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<tr>
<td>K&lt;sub&gt;p&lt;/sub&gt;</td>
<td>Permeability coefficient – (cm/h)</td>
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<tr>
<td>N&lt;sub&gt;obs&lt;/sub&gt;</td>
<td>Number of observations – (unit less)</td>
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<td>N&lt;sub&gt;par&lt;/sub&gt;</td>
<td>Number of parameters – (unit less)</td>
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<tr>
<td>P</td>
<td>Partition coefficient – (unit less)</td>
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<td>P&lt;sub&gt;F&lt;/sub&gt;</td>
<td>Partition coefficient from Franz diffusion cell study – (unit less)</td>
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<tr>
<td>P&lt;sub&gt;x&lt;/sub&gt;</td>
<td>Partition coefficient of compartment x – (unit less)</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>Q</td>
<td>Chemical amount – (mg)</td>
</tr>
<tr>
<td>SC</td>
<td>Stratum corneum</td>
</tr>
<tr>
<td>SS</td>
<td>Residual sum of squares</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermogravimetric analysis</td>
</tr>
<tr>
<td>T&lt;sub&gt;lag&lt;/sub&gt;</td>
<td>Lag time – (min)</td>
</tr>
<tr>
<td>W</td>
<td>Skin weight – (mg)</td>
</tr>
<tr>
<td>Z</td>
<td>Skin resistance – (s/cm²)</td>
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</table>
1 INTRODUCTION

The skin is the heaviest organ of the body (~15% of body weight) and its major function is to act as a barrier towards the outer environment, thus maintaining water homeostasis and protecting against chemical, biological and physical hazards (see e.g. Chuong et al. 20). However, the skin barrier is not perfect as a large number of chemicals do, to various degree, penetrate the skin (see e.g. Johanson et al. 45) to reach systemic exposure.

Several methods are available for studying dermal uptake of chemicals. In vivo methods, such as exposure chambers, give highly relevant information. However, these studies are often expensive, complicated to perform and can raise ethical considerations. In vitro methods, such as static and flow-through diffusion cells, are popular because of their ease to use and simple construction. However, large variations in reported dermal uptake for the same chemical is seen (e.g. $10^7$ for phenol, see e.g. Johanson et al. 45), indicating that the results are affected by choice of e.g. studied species, receptor fluid, analysis method and vehicle. Inter-laboratory variation has also been reported 19, further showing the need for a standardized approach when testing dermal penetration of chemicals in vitro.

The frequency and concentration of chemical exposures are often higher for workers than for the general population. Therefore, measures have been taken to reduce the health impact of chemical exposure, by introducing e.g. occupational exposure limits (OELs) and skin notations. OELs are legally binding, maximum tolerable concentrations which are set to protect humans from adverse effects from airborne substances. Skin notations are additional warnings indicating that the dermal uptake is high for that chemical.

The OELs, skin notations and also risk assessments (when skin exposure is present) rely on a strong, scientific basis. However, as a vast majority of the chemicals in use today lack dermal uptake data, this basis is weakened. Also, when dermal uptake data is present, it is found that the systemic uptake via the dermal route is equally high as that of inhalation for many chemicals (see e.g. Johanson et al. 45), further indicating that relevant, high quality dermal uptake data are needed.

The lack of, and seen large variations in, dermal uptake data, indicate the need for a new, standardized method for measuring dermal uptake of chemicals.

The aim of this project, as presented in this thesis, was to develop and validate the thermogravimetric analysis (TGA) method for measuring dermal uptake of chemical vapors.
2 DIFFUSION

The net transport of molecules from a region of higher molecular activity (i.e. number of free molecules) to one of lower activity is called diffusion. Diffusion is a passive process (i.e. no expenditure of energy) and is a result of the random movement of free molecules. Diffusion is a driving force in many biological and chemical processes, e.g. dermal absorption. The diffusion of molecules in a medium is correlated to several parameters, e.g. temperature, molecular size (or weight), molecular polarity and the viscosity of the medium (see e.g. Schaefer et al. 81). This molecular mobility in the medium is called the diffusion coefficient, \( D \).

**Fick’s first law of diffusion**

The net flow of molecules, i.e. flux, \( J \), through a homogenous medium is described by Fick’s first law of diffusion, as:

\[
J = -D \frac{\Delta A}{\Delta x}
\]

(2-1)

where \( \Delta A \) is the difference in activity and \( \Delta x \) is the distance in the medium. The negative sign indicates that the direction of the flux is opposite the activity gradient.

**Fick’s second law of diffusion**

The Fick’s second law of diffusion predicts the non-steady-state activity profile within the homogenous medium, as (for all three directions):

\[
\frac{\partial A}{\partial t} = D \left( \frac{\partial^2 A}{\partial x^2} + \frac{\partial^2 A}{\partial y^2} + \frac{\partial^2 A}{\partial z^2} \right)
\]

(2-2)

This equation (and the solution) is greatly simplified if diffusion is assumed to be present in only one direction (e.g. x-direction), as:

\[
\frac{\partial A}{\partial t} = D \frac{\partial^2 A}{\partial x^2}
\]

(2-3)

For a more extensive mathematical description of Fick’s laws, see e.g. Crank 22.

**Cross membrane flux**

Cross membrane flux will occur when the membrane separates a region of higher molecular activity, \( A_{\text{donor}} \), with one having lower activity, \( A_{\text{receptor}} \), Figure 2-1.

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![Figure 2-1](image)

**Figure 2-1** Membrane separating two regions, called donor and receptor, where the molecular activity in the donor is higher than the activity in the receptor. The activity gradient will cause the molecules to migrate through the membrane. At the top and bottom surfaces a partitioning of molecules will occur.
The partition coefficient, \( P_{\text{membrane:donor}} \), at the upper surface is defined as:

\[
P_{\text{membrane:donor}} = \frac{A_{\text{top}}}{A_{\text{donor}}}
\]

where \( A_{\text{donor}} \) is the molecular activity in the donor (region above the membrane) and \( A_{\text{top}} \) is the activity at the top layer of the membrane. Similarly, the partition coefficient at the lower surface is defined as:

\[
P_{\text{membrane:receptor}} = \frac{A_{\text{bottom}}}{A_{\text{receptor}}}
\]

where \( A_{\text{bottom}} \) is the molecular activity at the bottom layer of the membrane and \( A_{\text{receptor}} \) is the activity in the receptor (region below the membrane).

The cross membrane flux can now be calculated as:

\[
J = D \cdot \frac{A_{\text{top}} - A_{\text{bottom}}}{h} = D \cdot \frac{P_{\text{membrane:donor}} \cdot A_{\text{donor}} - P_{\text{membrane:receptor}} \cdot A_{\text{receptor}}}{h}
\]

where \( h \) is the membrane thickness. However, if \( A_{\text{receptor}} \) is very small compared to \( A_{\text{donor}} \), the cross membrane flux is simplified to:

\[
J = \frac{D \cdot P_{\text{membrane:donor}}}{h} \cdot A_{\text{donor}} = K_p \cdot A_{\text{donor}}
\]

where \( K_p \) is a called the permeability coefficient.

**Membrane resistance**

From the permeability coefficient, the resistance, \( Z \), of the medium is defined as:

\[
Z = \frac{1}{K_p}
\]

The total resistance, \( Z_{\text{tot}} \), when diffusion is through \( n \)-number of membranes, is defined for parallel membranes as:

\[
\frac{1}{Z_{\text{tot}}} = \frac{1}{Z_1} + \frac{1}{Z_2} + ... + \frac{1}{Z_n}
\]

For serial membranes the resistance is defined as:

\[
Z_{\text{tot}} = Z_1 + Z_2 + ... + Z_n
\]

**Using concentration as a measure of activity**

The molecular activity is related to the concentration, \( C \), by:

\[
A = \gamma \cdot C
\]

where \( \gamma \) is the activity coefficient. For highly diluted (close to ideal) solutions \( \gamma \) is close to 1 (i.e. higher activity). With increasing concentration \( \gamma \) is decreased due to an increased number of molecular interactions (i.e. lower activity).

In theory, as the diffusion coefficient is defined as the mobility of the molecules in the membrane, it is constant for all concentrations. However, the permeability and partition coefficients are not constant (when considering concentration) so different values are obtained for different concentrations.
In summary, when comparing two studies (1 and 2) performed (on the same type of membrane and substance) having very different concentrations; \( D_1 = D_2 \), \( K_{p1} \neq K_{p2} \) and \( P_1 \neq P_2 \), respectively.

When replacing activity with chemical concentration (g/cm\(^3\)) in the equations above, \( J \) is expressed in terms of g/cm\(^2\)/h, \( K_p \) in cm/h and \( D \) in cm\(^2\)/s, respectively.
3 THE SKIN
The skin is by weight (~7 kg) the largest organ of the body and contributes to 15% of the total body weight of an adult (see e.g. Schaefer et al. 81). It covers the entire body (~1.5-2 m²) and provides a barrier towards the outer environment, preventing water loss and protecting against mechanical, biological and chemical threats. Other important functions are thermo regulation, mechanical stability, UV-protection and communication (see e.g. Chuong et al. 20).

Figure 3-1 Drawing of the skin (image from Wikimedia 101).

Skin layers
The skin consists of three separate layers (from inner to outer); hypodermis, dermis and epidermis, Figure 3-1.

Hypodermis is the deepest layer of the skin and is a 1-2 mm thick region consisting mainly of fat, connective tissue (e.g. collagen), larger blood vessels and nerves. The more important functions are storage of energy, metabolism, mechanical stability and thermo regulation.

Dermis is the middle layer and is a 1-2 mm thick region consisting mainly of collagen, smaller blood and lymph vessels and nerves. Dermis provides strength and elasticity to the skin. Also, many appendages (e.g. hair follicles and sweat glands) that permeate the skin surface have their origin in dermis.
Epidermis is the top layer of the skin and is approximately 100 µm thick, but large variation are seen depending on body site where the thinnest is found on the eyelids (~50 µm) and the thickest is on the palms and soles (~1.5 mm). Epidermis contains both a viable and a non-viable (stratum corneum) region. The viable region consists mainly of keratinocytes (skin cells), melanocytes (pigmentation cells), Langerhans cells (immune cells) and Merkel cells (sensory reception cells). The composition of viable epidermis is 70% water, 15% proteins and 5% lipids (see e.g. Schaefer et al. 81).

Stratum corneum is the uppermost 10-30 cell layers (10-20 µm) of epidermis and consists of keratin filled cells (corneocytes) suspended in a (extra-cellular) lipid rich matrix. The composition of stratum corneum is 70% proteins, 15% lipids and 15% water (see e.g. Schaefer et al. 81), thus there is a large water gradient across the stratum corneum 100.

3.1 THE SKIN AS A DIFFUSION BARRIER
When physically removing the stratum corneum (by e.g. tape stripping) a dramatic increase in permeability for water 83 and other compounds 82 is observed, indicating that the stratum corneum is the rate limiting barrier for dermal penetration 72.

The organization of stratum corneum can be seen as a brick wall where corneocytes represents the bricks and inter-cellular lipid matrix the mortar 64, Figure 3-2. The only continuous route through stratum corneum is therefore via the inter-cellular pathway, and the length of this pathway is greatly increased (ten-fold 94) by the corneocyte overlap.

![Figure 3-2](image_url)

Figure 3-2 Molecules that diffuse through the stratum corneum will take a long and tortuous pathway through the inter-cellular lipid matrix. Please note that the inter-cellular space in this picture is heavily exaggerated.

The inter-cellular matrix is considered to be a diffusion pathway for lipophilic substances 67 whereas water diffusivity through the same pathway is low 35, 36, 48, 87.

A second route of diffusion through stratum corneum is via the intra-cellular pathway, i.e. through the corneocytes. However, the cells are not adjacent so the molecules must diffuse through the inter-cellular lipid matrix in order to reach the next corneocyte.

A third pathway through stratum corneum is via the appendages (hair follicles, sweat glands and sebaceous glands). Although the appendages are open, direct pathways through the stratum corneum to the viable epidermis, the total flux through them will be rather low as they only account for 0.1-1% of the surface area of the skin (see e.g. Schaefer et al. 81).

3.2 SKIN DIFFUSION MODELS
There are several types of available models for predicting percutaneous penetration of substances (see e.g. Riviere 89), and the choice of model depends on the purpose. The more common models are;
Pharmacokinetic models are used when studying the time course of chemical absorption, distribution, metabolism and excretion in the body. When dermal absorption is modeled, the skin is often described using one or two compartments. These compartments could be physiologically relevant (i.e. modeling stratum corneum, epidermis or dermis), however the compartments are considered well stirred thus only average concentrations are calculated.

Diffusion models are based on the Fick’s second law of diffusion and describe the diffusion through the skin as a function of time and distance. The models have physiologically relevant compartments (e.g. inter-cellular lipid matrix, corneocytes, stratum corneum) and both one compartment models and two compartment models have been derived. The models describe the diffusion through the skin in great detail.

Structure-activity based models, are derived from large datasets and predict e.g. permeability and partition coefficients by using chemical parameters, e.g. molecular weight and octanol:water partition coefficient.
4 DIFFUSION CELLS
When studying dermal penetration of chemicals, *in vivo* data is of course preferred, however, the experiments are not always feasible, due to e.g. cost, study design or for ethical reasons. Therefore, several *in vitro* methods have been developed for dermal penetration studies, the most common being the diffusion cells; the static (Figure 4-1) and flow-through diffusion cells (also called the Franz and Bronaugh diffusion cells, respectively) (see e.g. Smith *et al.* 92 for further description).

![Diffusion Cell Diagram](image)

**Figure 4-1** A static diffusion cell with a water jacket. The skin is clamped between the two compartments (donor and receptor). The donor is filled with chemical and the receptor is filled with a receptor medium. The receptor medium is sampled at defined times and analyzed by e.g. gas chromatography. The water jacket maintains the receptor medium at a constant 32°C. Note the arrows to the left indicating the flow of water to/from the jacket.

The basic idea of the two cell types is very similar, as a piece of skin is clamped between two compartments (called donor and receptor). The donor compartment is filled with a known concentration of the chemical of interest and the receptor compartment is filled with a suitable receptor medium. In the flow-through cells, the receptor medium is continuously replaced with fresh medium, whereas in the static cells the receptor medium is left unchanged.

Throughout the exposure, the chemical will partition into the skin, diffuse through the skin and finally partition into the receptor fluid (if correctly chosen receptor fluid). At defined times, samples are taken from the receptor fluid and the concentration of the chemical is measured by e.g. gas chromatography.

As for all *in vitro* cell studies, the choice of receptor medium is crucial. The use of water, physiological saline or a phosphate buffered saline (PBS) solution are suitable for hydrophilic chemicals, however for lipophilic chemicals the OECD guidelines 70 recommends 6% PEG-20 in a phosphate buffered saline (PBS) solution.
Thermogravimetric analysis (TGA), Figure 5-1, is a technique which measures weight as a function of temperature and time. Essentially, the TGA is a high precision micro-balance with the sample located in a temperature-controlled atmosphere. The TGA is a multi-purpose instrument which is primarily used for e.g.; measuring thermal stability of materials (e.g. glass transition temperatures), polymer flammability and predicting product lifetime.

Figure 5-1 A vertical furnace/horizontal purge TGA. The sample is placed onto a platinum pan (drawn as white pan) and inserted into the furnace (black cylinder) of the TGA. An airflow enters the furnace (left arrow) and is extracted by the fume extractor arm (right arrows).

Many TGA instruments are based on a null-balance principle, Figure 5-2, i.e. two platinum pans (the tare and sample pans) are attached to each side of a balance arm. A well defined weight (tare weight) is placed in the tare pan and the sample (which is of lesser weight than the tare weight) is placed in the sample pan. The level of the balance arm is continuously measured by a sensor, and the unbalance is corrected by a meter movement mechanism. When a correct balance is achieved, the amount of current used to adjust the balance arm is directly proportional to the weight of the sample.
The sample pan is placed inside the furnace cylinder, Figure 5-3, and hangs from a fine metal thread close to the temperature probe. Two flows of gas enter the furnace; a horizontal flow (air) and a vertical flow (nitrogen). The vertical flow (called purge and should equal 10% of the horizontal flow) is needed to ensure the safety of the sensitive electronics located above the furnace. At the bottom of the furnace, there is an outlet (normally closed) for maintenance purposes.
6 AIMS OF THIS THESIS
The overall aim of this thesis was to develop the new method of measuring dermal absorption of chemical vapor using the thermogravimetric analysis (TGA) technique.

The more specific aims were to:

I. To assess that the TGA technique allows reliable recordings of the minute weight changes of skin exposed to chemical vapor,

II. develop a high-precision, computer controlled chemical vapor generating system to be used in TGA measurements of dermal uptake of chemical vapor,

III. derive a TGA specific solution to the Fick’s second law of diffusion to allow the analysis of the obtained weight curves from exposing skin to chemical vapor,

IV. to validate the TGA method by comparison against Franz diffusion cell data for a number of volatile chemicals.
7 MATERIALS AND METHODS

7.1 ETHICAL APPROVAL
No ethical permissions were needed since skin samples were obtained from pigs that had not been bred for research purposes and that died of natural causes.

7.2 CHEMICALS
Cyklohexanone (CAS 108-94-1, 99% purity), n-butyl acetate (CAS 123-86-4, >99% purity), methanol (CAS 67-56-1, 99% purity) and 2-propanol (CAS 67-60-1, >99.5% purity) were obtained from Sigma-Aldrich (Steinheim, Germany). n-Butanol (CAS 71-36-3, 99.5% purity), n-hexane (CAS 110-54-3, ≥98% purity) and m-xylene (CAS 1330-20-7, ≥99% purity) were obtained from Merck (Darmstadt, Germany). Ethyl benzene (CAS 100-41-4, ≥98% purity) and styrene (CAS 100-42-5, ≥99% purity) were obtained from Fluka (Buchs, Switzerland). Toluene (CAS 108-88-3, >99.5% purity) was obtained from Kebo Lab (Stockholm, Sweden). Phosphate buffered saline (PBS) was prepared from phosphate standard concentrate (1000-3) obtained from Sigma-Aldrich and sodium chloride (analytical grade) was obtained from Merck.

7.3 SKIN
Piglets (Duroc), that were either stillborn or died (of natural causes) within three days of birth, were obtained from local, commercial breeders. Selected pieces of full thickness skin from ear, back and flank were gently removed using forceps and scalpel. Each piece was uniquely numbered, wrapped in aluminum foil and polyethene film (with double folded ends) and placed in a freezer (−20°C).

7.4 ASSESSMENT OF DERMAL ABSORPTION BY TGA (STUDY I)

Skin separation by trypsin
Thawed, full thickness pig skin from the back of the ear, were cut into round pieces (ø8 mm) using a biopsy punch, Figure 7-1. The skin pieces were placed, with the stratum corneum side up, in a Petri dish containing a soaked (0.5% bovine trypsin in PBS buffer at pH 7.4) glass fiber filter paper, Figure 7-2. The Petri dish was thereafter placed in a refrigerator (4°C).

After approximately 24 h, epidermal sheets were gently peeled off using forceps. The epidermal pieces were placed in an aqueous inhibitor solution (0.005% trypsin inhibitor in PBS) for approximately 10 minutes, washed in saline for approximately 30 minutes, wrapped in aluminum foil and polyethene film (with double folded ends) and placed in a freezer (−20°C).

Skin preparation
A piece of frozen trypsinated pig skin was placed to thaw in a physiological saline containing Petri dish. After approximately five minutes, the TGA platinum pan, Figure 7-3, was filled with saline and the skin was placed on the obtained saline meniscus. The stratum corneum side of the skin was found by turning the skin over, thus the skin followed the meniscus curvature when stratum corneum was upward. Excess water was gently removed using cotton swab. Thereafter, the skin was dried by flowing dry, clean air over the skin for approximately 30 seconds.

The skin thickness was measured (if possible) after the experiment using a micrometer (293-661-10, Mitutoyo, Japan).
Figure 7-1 Full thickness skin is placed on a plastic cutting board (with the *stratum corneum* side up) and round pieces (Ø8 mm) are obtained using a biopsy punch. Thereafter, the pieces are placed in a Petri dish (to the left) that contained trypsin solution.

Figure 7-2 Round pieces of full thickness skin are placed (with *stratum corneum* side up) on a glass fiber filter paper that is soaked in trypsin solution.
Figure 7-3 In the TGA studies, pig epidermis (Ø8 mm) is placed in a platinum pan with the stratum corneum side up.

**TA Instruments TGA 2050**
The TGA 2050 (TA Instrument Inc., New Castle, DE, USA), Figure 5-1, is a microbalance with high sensitivity (0.2 µg) and high thermal stability (standard deviation 0.01°C at 35°C). High capacity ceramic heaters are mounted inside the furnace, enabling temperatures above 1000°C.

The supplied TGA-specific computer software, Thermal Advantage (v 1.1A, TA Instrument Inc), facilitates temperature programming and data collecting. An isothermal, 35°C, program was used for all TGA measurements.

**TGA experiments**
Round pieces (ø8 mm) of pig epidermis were placed onto a platinum pan and inserted into the TGA furnace where the temperature (35°C) and humidity (45% RH) were maintained at constant levels. The skin was intermittently exposed to known concentrations of chemical vapor while the weight of the skin was recorded three times per second.

**Calculations**
The concentration of the chemical in the skin, \( C_{\text{skin}} \) (g/cm³), is calculated by:

\[
C_{\text{skin}} = \frac{\Delta W_{\text{skin}} \cdot \rho}{W_{\text{base}}} \quad (7-1)
\]

where \( \Delta W_{\text{skin}} \) is the weight increase of the skin at equilibrium (µg), \( W_{\text{base}} \) is the skin weight prior exposure (µg) and \( \rho \) is the skin density (g/cm³). For convenience, the density of the skin was chosen to be 1 g/cm³.

The skin:air partition coefficient, \( P_{\text{skin:air}} \), is defined as:

\[
P_{\text{skin:air}} = \frac{C_{\text{skin}}}{C_{\text{TGA}}} \quad (7-2)
\]

where \( C_{\text{TGA}} \) is the concentration of the chemical in the TGA furnace (g/cm³) and is considered to be constant.
7.5 VAPOR GENERATING SYSTEM (STUDY II)

7.5.1 Evaporation

Evaporation is defined as the spontaneous process of molecules leaving the liquid phase and entering the gas phase, and occurs at the liquid:air contact surface (see e.g. Zumdahl \(^{103}\)). The evaporation process is endothermic and the rate of evaporation is correlated to the temperature of the liquid.

The amount of evaporating chemical is greatly increased by expanding the liquid:air contact surface, which is done e.g. in gas wash bottles where air is bubbled through the liquid.

7.5.2 Gas wash bottle

The evaporation process is temperature dependent. Therefore, as a stable vapor concentration is desired, the maintaining of a constant liquid temperature is crucial. This is solved by placing the bottles in a thermo regulated water bath. Stainless steel gas wash bottles were obtained from a local workshop (Workshop at Karolinska Institutet, Stockholm, Sweden). Stainless steel bottles were preferred instead of glass bottles as steel has higher thermal conductivity and standard Swagelock fittings (i.e. nuts and bolts) could be used.

![Figure 7-4 Schematic picture of gas wash bottle. The clean, dry air is bubbled through the liquid (blue) before leaving the bottle.](image)

![Figure 7-5 Tailor-made stainless steel gas wash bottle.](image)

Calculations

The saturation concentration of chemical in air (at 25°C) was calculated from:

\[
C_{\text{air}} = 0.455 \cdot \frac{MW}{24.5} \cdot \frac{P_{\text{vap}}}{101.3}
\]

(7-3)

where MW is the molecular weight of the chemical (g/mol), \(P_{\text{vap}}\) is the partial vapor pressure of the chemical (kPa) at 25°C, 24.5 is the molar volume of gas at 25°C (l/mol) and 101.3 is the normal atmospheric pressure at 25°C (kPa).

The vapor pressures of the chemicals were obtained from the “Handbook of chemistry and physics” \(^{86}\).
7.5.3 Gas wash bottle performance

The performance of a cyclohexanone containing gas wash bottle was measured using a photo ionization detector (PID, Micro-TIP MP-100, Photovac Inc., Waltham, MA, USA), Figure 7-6.

![Figure 7-6 Microtip photo ionization detector. The sample enters through the inlet to the right and leaves through the outlet (plastic tube).](image)

Mass flow controllers were used to dilute the cyclohexanone vapor to the linear range of the photo ionization detector, Figure 7-7.

![Figure 7-7 Clean, dry air is bubbled through a cyclohexanone containing gas wash bottle (GWB) and diluted before reaching the photo ionization detector (PID) where the concentration of cyclohexanone in the air is measured.](image)

A two-point calibration was performed; 0 ppm (using clean air) and 100 ppm cyclohexanone in clean air (from a gas-tight polyethylene aluminum bag).

7.5.4 Airflow control

**Mass flow controllers**

Digital mass flow controllers are commonly used for high precision controlling and monitoring of airflows. Therefore, two digital mass flow controllers were obtained (DFCS-010005, Aalborg, Orangeburg, NY, USA), Figure 7-8, having a 0-500 ml/min range.
**Magnetic valves**

Magnetic valves are commonly used for directing the flow of liquid or gas.

A low voltage system is required as highly flammable chemical vapors will be present. Therefore, solenoid driven, 24 V=, spring released 2/2 valves, Figure 7-9, (where the first 2 is the number of ports, i.e. in and out, and the second 2 is the number of available positions, i.e. open and close) were used, Figure 7-10. Two types of coated valves were obtained; *standard coated* (for contact with air and water vapor, model 125317L, Bürkert GmbH, Ingelfingen, Germany) and *non-corrosive Teflon coated* (for contact with chemical vapor, model 150830, Bürkert GmbH).

![Figure 7-8 A Aalborg mass flow controller.](image1)

![Figure 7-9 Circuit function (closed position) of the solenoid driven, spring released 2/2 magnetic valve.](image2)

![Figure 7-10 A Bürkert magnetic valve.](image3)

**Electronic interface**

An electronic interface was constructed to facilitate computer control of the magnetic valves, having the following functions:

- communication via the printer ports of the computer,
- one electronic relay per magnetic valve, converting the 5 V= delivered by the printer port to the 24 V= needed by the magnetic valves,
- one d-latch (electronic memory circuit) per magnetic valve, ensuring stability and protection of the computer printer port.

Later, a computer I/O-card (PCI-1751, Advantech, Taiwan) was obtained, replacing the communication via the printer ports.

**Computer software**

Two dedicated computer programs were created (in Borland C++ Builder 6.0), to allow operation of the mass flow controllers, Figure 7-11, and the vapor generating system, Figure 7-12 and Figure 7-13. The software allows creation and automatic execution of running schedules, facilitating precise timing and long time runs.
Figure 7-11 Screenshot from the mass flow controller computer software, showing the actual and desired flow rates of the two airflows entering the vapor generating system.

Figure 7-12 Screenshot from the magnetic valve controlling computer program, showing the creation of a running schedule.
7.6 DEVELOPMENT OF TGA-SPECIFIC DIFFUSION MODEL (STUDY III)

Calculations

The upper and lateral surfaces of the skin are in direct contact with the ambient air in the TGA experiments. However, to simplify the TGA-specific solution to Fick’s second law, the horizontal diffusion (entering the skin from the sides) is neglected as the area of the lateral surface of the skin is considerably smaller than the area of the upper surface. No net flux will occur across the bottom surface of the skin (lower boundary) as it is in direct contact with the platinum pan, Figure 7-14.

\[ C_{\text{skin}}(x=0) = P_{\text{skin:air}} \cdot C_{\text{TGA}} \]

\[ \frac{dC_{\text{skin}}(x=h)}{dx} = 0 \]

Figure 7-14 Schematic drawing of skin on the platinum pan placed in the TGA furnace.

The following parameters are known (or measured) prior the TGA experiments; the skin thickness \( h \) (cm), the exposed area of the skin \( A \) (cm\(^2\)) and the concentration of chemical in the ambient air, \( C_{\text{TGA}} \) (mg/cm\(^3\)), Figure 7-14.

The boundary conditions of the skin are:

\[ C_{\text{skin}}(0,t) = P_{\text{skin:air}} \cdot C_{\text{TGA}} \]  
\[ \frac{\partial C_{\text{skin}}(h,t)}{\partial x} = 0 \]
The initial conditions of the skin, at start of exposure to chemical vapor, are:

\[ C_{\text{skin}}(0,0) = P_{\text{skin\_air}} \cdot C_{\text{TGA}} \quad (7-6) \]

\[ C_{\text{skin}}(x,0) = 0 \quad 0 < x \leq h \quad (7-7) \]

If the skin thickness was unknown, it was calculated by:

\[ h = \frac{W_{\text{base}}}{\rho \cdot A} \quad (7-8) \]

where \( W_{\text{base}} \) is the weight of the skin (mg) at 45% RH and \( \rho \) is the skin density (0.22 g/cm³). The skin density was obtained from TGA experiments (n=21) and calculated from the base weight and volume of the skin.

**The Akaike Information Criterion**

When fitting a mathematical model to a set of data, an increased number of model parameters will provide a better fit, i.e. decrease the residual sum of squares, Figure 7-15. However, increasing the number of parameters results in a more unspecific model.

The Akaike Information Criterion (AIC) is a model selecting tool, that compares a models goodness of fit to the number of model parameters used (see e.g Burnham et al. 18). The AIC value can be calculated by:

\[ AIC = N_{\text{obs}} \cdot \ln \left( \frac{SS}{N_{\text{obs}}} \right) + 2 \cdot N_{\text{par}} \quad (7-9) \]

where \( N_{\text{obs}} \) is the number of data-points, \( SS \) is the residual sum of squares and \( N_{\text{par}} \) is the number of parameters in the model. When comparing two models, the one with lowest AIC is preferred, Figure 7-15.

![Figure 7-15 The calculated AIC-value (● – left axis) and sum of squares (○ – right axis) for a TGA specific diffusion model, curve-fitted to a TGA obtained data set, plotted against the number of used parameters in the model, \( N_{\text{par}} \). As the number of parameters increase, the fit improves (seen as a decrease of sum of squares). The lowest AIC-value (for this specific data set) is obtained for ten parameters, i.e. a five compartment model, as seen in the magnified area.](image-url)
7.7 VALIDATE TGA METHOD AGAINST FRANZ CELLS (STUDY IV)

7.7.1 Diffusion cell studies

*Skin separation by dermatome*

Thawed full thickness pig skin (from back and flank) was mounted, with *stratum corneum* side up, onto soft polyethene holders using a staple gun, Figure 7-16 and Figure 7-17.

Figure 7-16 Thawed, full thickness pig skin mounted onto a polyethene plastic holder, before storage in the freezer.

Thereafter, a polyethene lid was placed onto the skin and attached onto the bottom piece by screws, Figure 7-16, thus applying pressure to the skin piece. The skin was stored in a freezer (-20°C) for 24 h and thereafter dermatomed, Figure 7-18.

Figure 7-17 A Padgett dermatome, model C, and full thickness pig skin mounted onto polyethene plastic skin holders. The skin is attached by staples (arrows) and the holder is mounted onto a polyethene board.
Diffusion cell experiments
Full or split-thickness skin was used for the Franz diffusion cell studies. The skin thickness was measured using a micrometer (293-661-10, Mitutoyo, Japan) and skin integrity was checked by measuring cross membrane resistance (over 0.64 cm², Fluke 111, Fluke Corporation, Everett, WA, USA) when the skin was mounted in a Franz diffusion cell having physiological saline in donor and receptor compartment. The skin pieces were thereafter placed in physiological saline containing Petri dishes for over-night storage in a refrigerator (+4°C).

Six jacketed static Franz cells (orifice diameter 9 mm corresponding to a skin exposure area of 0.64 cm², volume 5.0-5.4 ml, model number 4G-01-00-09-05, Permegear, Bethlehem, PA, USA) were placed on a magnetic stirrer (HP 6 Variomag, H+P Labortechnik, Munich, Germany) and kept at 32°C by circulating water from a thermostatted water bath (21 AT, Heto, Allerød, Denmark). After adding ultra-sound degassed receptor medium (6% PEG-20 in PBS) to the receptor compartment, skins were mounted (stratum corneum side up) and left to acclimatize. After one hour, 1 ml of chemical was added to the donor compartment and the cells were capped using a glass stopper. Measures were taken to assure that the chemical in the donor compartment was in excess and the receptor medium was well stirred (using a Teflon coated magnetic stirrer) during the whole experiment.

Aliquots of 50 µl of receptor fluid were taken, using a 50 µl gas-tight syringe (004250, SGE, Victoria, Australia), at defined times (h:mm; 0:00, 0:10, 0:20, 0:30, 0:40, 0:50, 1:00, 1:20, 1:40, 2:00 and thereafter every 30 min). The samples were transferred to gas-tight headspace vials and stored in a refrigerator (+4°C) for later analysis.
**Computer software**

A computer program was created (in Borland C++ Builder 6.0) to aid the operator during the diffusion cell experiments, Figure 7-19 and Figure 7-20, facilitating precise timing of sampling.

**Figure 7-19** Screenshot from the diffusion cell program, showing the sampling schedule for 2−propanol.

**Figure 7-20** Screenshot from the diffusion cell program, showing the information the operator needs during the experiments for precise timed sampling.
**Calculations**

The following parameters are known (or measured) prior the Franz diffusion cell experiments; the skin thickness $h$ (cm), the exposed area of the skin $A$ (cm$^2$) and the concentration of the chemical in the donor compartment $C_{\text{donor}}$ (mg/cm$^3$), Figure 7-21.

![Diagram of skin mounted in a vertical in vitro diffusion cell](image)

Figure 7-21 Schematic figure of skin mounted in a vertical *in vitro* diffusion cell, as seen in Figure 4-1.

The concentration of the chemical in the receptor compartment will be very low compared to the concentration of the chemical in the donor. The cross membrane concentration, $\Delta C = C_{\text{donor}} - C_{\text{receptor}}$, is therefore assumed to equal $C_{\text{donor}}$.

The lag-time is defined as the time taken for the chemical to reach the receptor medium, i.e. to penetrate the skin. However, lack of measurement sensitivity and non-continuous measurement of receptor fluid makes it impossible to measure the exact time of penetration. Therefore, the apparent lag-time, $t_{\text{lag}}$, is used and is defined as the time where the tangent to the steady-state part of the absorbed amount curve intercepts the x-axis, Figure 7-22.

![Graph of cumulative absorbed amount of toluene](image)

Figure 7-22 Cumulative absorbed amount of toluene in receptor fluid. Samples analyzed by gas chromatography. The dotted line represents the steady-state slope of the absorbed amount curve.
With a known lag-time, the diffusion coefficient \( D \) (cm\(^2\)/s) is calculated as:

\[
D = \frac{h^2}{6 \cdot t_{lag}} \quad (7-10)
\]

From the slope of the steady-state part of the cumulative absorbed amount, \( Q \) (mg), the steady-state flux, \( J_{ss} \) (µg/cm\(^2\)/h), can be calculated as:

\[
J_{ss} = \frac{1}{A} \frac{dQ}{dt} \quad (7-11)
\]

Thereafter, the permeability coefficient, \( K_p \) (cm/h), is calculated as:

\[
K_p = \frac{C_{donor}}{J_{ss}} \quad (7-12)
\]

The octanol:water partition coefficients were obtained from the computer software KowWin \(^5^4\) using data published by Hansch \textit{et al.} in 1995.

**Sample analysis**

The samples were analyzed by means of gas chromatography (GC, Clarus 500, Perkin Elmer, Waltham, MA, USA). The column (Chrompack 7583, Varian, Palo Alto, CA, USA) was 10 m long and Poraplot Q coated.

The column temperatures and retention times were: 180°C and 3.1 min for n-butanol, 175°C and 5.1 min for n-butyl acetate, 190°C and 4.8 min for cyclohexanone, 220°C and 3.6 min for ethyl benzene, 130°C and 3.8 min for n-hexane, 70°C and 2.1 min for methanol, 130°C and 6.8 min for 2-propanol, 220°C and 3.1 min for styrene, 180°C and 2.3 min for toluene, 190°C and 3.3 min for m-xylene, respectively.
8 RESULTS AND DISCUSSIONS

8.1 ASSESSMENT OF DERMAL ABSORPTION BY TGA (STUDY I)

8.1.1 Results

Trypsinated pig epidermis (ø8 mm) was placed in a platinum pan and inserted into the TGA furnace. A carefully controlled stream (90 ml/min) of humidified (45% RH) air was passed over the skin while the skin weight was continuously measured (three times per second). Dermal exposure to chemical vapor was achieved by adding known concentrations of chemical vapor to the air stream.

The weight change of the skin piece was readily recorded prior, during and after exposure to methanol, 2-propanol, toluene and water, respectively. As expected, upon addition of chemical vapor, the weight of the skin increased until an equilibrium was reached between air and skin (which was seen as a plateau on the recorded weight curve), Figure 8-1. After removal of the chemical vapor, the weight of the skin decreased and later reached (the same or a new) baseline level, Figure 8-1.

Figure 8-1 The recorded weight of a piece of pig epidermis during exposure to methanol, water and methanol, using the TGA method.
Figure 8-2 The relative weight change of a piece of trypsinated pig epidermis exposed to chemical vapor (starting at 10 min) with a 180 minute duration. From the top; water, 2-propanol, methanol and toluene.

8.1.2 Discussion
The weight change of pig epidermis prior, during and after exposure to chemical vapor, was readily recorded. When comparing the recorded skin weight curves, Figure 8-2, it was noticeable that the shape of the weight curve and the total weight increase were different for each studied chemical, reflecting the differences in chemical diffusivity and skin:air partition coefficient, respectively.

The TGA method is ideally suited for measuring small weight changes of skin as the temperature of the sample is so carefully controlled.

8.2 VAPOR GENERATING SYSTEM (STUDY II)
8.2.1 Results
Vapor generating system
Seven custom made stainless-steel gas wash bottles were placed in heated water bath (25 ± 0.05°C), Figure 8-3. Chemical vapor was created by flowing clean, dry air through the chemical containing bottles. Magnetic valves were placed before and after every gas wash bottle to direct the airflow and to prevent back-flow.

The vapor generating system was built as two parallel systems, were the first two bottles can produce water vapor and the other five bottles can produce water or chemical vapor, Figure 8-4. Therefore, the skin in the TGA furnace can be exposed to both water and chemical vapor at the same time, Figure 8-5, maintaining skin hydration. The airflow through each system, 45 ml/min, is maintained by a computer-controlled mass flow controller. The airflows are combined after the gas wash bottles (giving a total of 90 ml/min) and directed to the TGA furnace. The chemical concentration in the airflow entering the TGA is therefore 50% of its saturation concentration.
Figure 8-3 Overview of the vapor generating system. Clean air enters to the left (black arrows) and is directed via magnetic valves (black boxes) to flow through the desired stainless steel gas wash bottle (gray cylinders). Tubing is insulated and heated using thermo-regulated heating cable. The vapor containing air leaves the system (white arrows), and enters the TGA, to the right.

All downstream connectors and tubing are made of stainless steel. They are also insulated and heated to 30 ± 1°C with a thermostatted (model 16150, Termonic, Skellefteå, Sweden) heating cable (Euroheat, 11 Ω/m) to prevent condensation and adsorption of chemical vapor.

Figure 8-4 Schematic drawing of the two parallel systems of the vapor generating system. Circles represent the gas wash bottles, where 1 and 3 are empty, 2 and 4 contain water and 5-7 contain the chemicals of interest. Magnetic valves are placed before and after every gas wash bottle. Mass flow controllers are placed at the inlet of each system for airflow maintaining purposes. In the figure, bottles 2 and 7 are opened.
Figure 8-5 Changes in chemical vapor concentration and relative humidity over time, as controlled by the vapor generating system. The numbers (1, 2, 3, 1) represent three different chemicals.

Gas wash bottle performance
The performance of a gas wash bottle was measured with a photo ionization detector using cyclohexanone as test substance. The average measured concentration was 5881 ± 103 ppm, corresponding to 100.2 ± 1.7% of the calculated saturated concentration and 50% and 90% saturation was reached after 1.6 ± 0.3 s and 5.4 ± 0.8 s, respectively. For further information see chapter 16.

8.2.2 Discussion
Vapor generating system
The computer controlled vapor generating system is ideal for TGA measurements as the concentrations of chemical vapor in air are high (i.e. close to saturation concentration) and stable. The precise timing of the created computer software facilitates mixed exposures and long time studies.

During the TGA measurements, a cyclic saw-toothed noise was seen on the recorded weight curves, having a period of approximately ten minutes. After observing the system it was found that the period of the noise corresponded to the on/off times of the thermo-regulator (connected to the heating cable), as the regulator was set to maintain 30°C with minimum and maximum intervals being 29 and 31°C, respectively. The altered temperature (and pressure) of the airflow, is probably detected by the sensitive TGA scale (and seen as noise), thus the noise is not due to electrical disturbance.

A temperature gradient is created to avoid any condensation and adsorption in the tubes, thus the water bath is maintained at 25°C, the tubes at 30°C and the temperature inside the TGA furnace is maintained at 35°C, respectively.

The saturation of chemical in the air leaving the vapor generating system is 50%, however inside the TGA furnace the air is diluted with the nitrogen purge gas (9 ml/min). Thus the resulting level of saturation in the furnace is 45.5% (=45/99 ml/min).

Gas wash bottle performance
The measured concentration of cyclohexanone in air leaving a gas wash bottle was very close to its theoretical saturation concentration. Cyclohexanone was chosen as test substance as it has a relatively low vapor pressure (~590 Pa at 25°C). As the rate of evaporation is correlated to the vapor pressure, all chemicals having a higher vapor pressure (than that of cyclohexanone) will have at least the same level of saturation.
8.3 DEVELOPMENT OF TGA-SPECIFIC DIFFUSION MODEL (STUDY III)

8.3.1 Results

**TGA-specific solution to Fick’s second law of diffusion**

With the TGA method, round pieces (ø8 mm) of pig epidermis are placed in a platinum pan and exposed to known concentrations of chemical vapor, \( C_{TGA} \), while the weight of the skin is recorded three times per second.

To obtain diffusion parameters from the recorded weight curves, a TGA-specific solution, \( W_{skin}(t) \), to Fick’s second law of diffusion was derived, see chapter 14, which equals:

\[
W_{\text{skin}}(t) = W_{\text{base}} + P_{\text{skinc}} \cdot C_{TGA} \cdot A \cdot h \left( 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \frac{e^{-(\frac{2(n+1)^2 \pi^2 h^2}{4h^2})}}{(2n+1)^2} \right) \quad (8-1)
\]

A TGA-specific, multi compartment diffusion model was created by assuming \( m \) number of parallel diffusion pathways through the skin, described as:

\[
W_{\text{skin}}(t) = W_{\text{base}} + C_{TGA} \cdot h \cdot \sum_{i=1}^{m} A_i \cdot P_{s\text{kin}i} \cdot \left( 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \frac{e^{-(\frac{2(n+1)^2 \pi^2 h^2}{4h^2})}}{(2n+1)^2} \right) \quad (8-2)
\]

where each compartment has its own set of diffusion, \( D_i \) and partition, \( P_i \), coefficients, respectively.

**Curve-fitting and model selection**

The multi compartment, TGA-specific skin model, equation 8-2, was fitted to TGA obtained skin weight curves, Figure 8-6, for n butyl acetate, methanol, 2-propanol and toluene. Best fits were obtained using the “curve fit” procedure in Berkeley Madonna software (v.8.3.9) by adjusting the diffusion and skin:air partition coefficients.

![Figure 8-6 TGA specific two compartment model (thick black line) fitted to TGA obtained weight curve (uneven black line) from dermal exposure to known concentration of toluene vapor. The first compartment (- - -) is fast having low capacity whereas the second compartment (· · ·) is slow having high capacity.](image-url)
By applying the Akaike Information Criterion (AIC) to the fitted models, the sufficient number of compartments was calculated (in MS Excel) for each weight curve. The compartment distribution for all of the studied chemicals (n=70) was; one – 14%, two – 58%, three – 13%, four – 11% and five – 4%. Thus the two compartment model was found to be most sufficient.

The resulting two compartment model was defined as:

$$W_{\text{skin}}(t) = W_{\text{base}} + W_1(t) + W_2(t)$$  \hspace{1cm} (8-3)

where \(W_1(t)\) and \(W_2(t)\) are defined as:

$$W_1(t) = P_1 \cdot C_{\text{TGA}} \cdot A \cdot h \cdot \left(1 - \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{(2n-1)^2} e^{-\frac{(2n-1)^2 \pi^2 D_h}{4h^2}}\right)$$  \hspace{1cm} (8-4)

$$W_2(t) = P_2 \cdot C_{\text{TGA}} \cdot A \cdot h \cdot \left(1 - \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{(2n)^2} e^{-\frac{(2n)^2 \pi^2 D_h}{4h^2}}\right)$$  \hspace{1cm} (8-5)

\[8.3.2\] \textbf{Discussion}

\textit{TGA-specific solution to Fick’s second law of diffusion}

In the TGA studies, the skin lays horizontally on a pan while being exposed to chemical vapor, thus dermal absorption will occur from both the upper and lateral surfaces of the skin. However, the area of the upper surface \((A=\pi r^2 \approx 50 \text{ mm}^2)\) is significantly larger than the area of the lateral surface \((A=2\pi rh \approx 1.3 \text{ mm}^2)\), thus the lateral (horizontal) is of minor importance compared to vertical diffusion (~2.5%).

An alternative solution to Fick’s second law of diffusion, having the same boundary conditions as the TGA-specific scenario, is obtained by considering a plane sheet which is exposed to a known constant concentration on both the upper and lower sides. When studying the junction that horizontally divides the sheet into two equally large planes, there will be no net diffusion across (as the concentrations on each side are always equal). The solution of the upper half will equal our derived TGA-specific solution.

A TGA-specific solution to Fick’s second law has previously been published \(^{55}\), however when studying the published equation, it equals the solution to a plane sheet having chemical exposure to the upper and lower sides. This will result in a four times higher diffusion coefficient than the solution assuming no net flux cross the bottom surface.

\textit{Curve-fitting and model selection}

By using the Akaike Information Criterion, it was found that a two compartment parallel diffusion model was sufficient to describe the TGA obtained weight curves.

When studying the compartment parameters, it was found that the first compartment had a significantly higher permeability coefficient than the second compartment. However, the second compartment had a higher capacity than the first one.
8.4 VALIDATE TGA METHOD AGAINST FRANZ CELLS (STUDY IV)

8.4.1 Results
TGA and Franz diffusion cell studies were preformed on n-butanol, n-butyl acetate, cyclohexanone, ethyl benzene, n-hexane, methanol, 2-propanol, styrene, toluene and m-xylene, respectively.

TGA method
The skin weight changes during exposure to the studied chemicals were all readily recorded.

The TGA-specific two compartment diffusion model was fitted to all of the recorded weight curves, and the model parameters \(D_1, D_2, P_1\) and \(P_2\) were estimated, Figure 8-7.

![Figure 8-7](image)

Figure 8-7 Diffusion coefficients (mean ± 95% CI) (○=\(D_1\), ●=\(D_2\)) obtained from fitting the TGA-specific two compartment model to skin weight curves, plotted against the octanol:water partition coefficients of the chemicals.

During these experiments, a cyclic saw-toothed noise was seen on the recorded weight curves, as previously mentioned in chapter 8.2.2. This noise would affect the curve-fitting procedure in Berkeley Madonna and thereby altering the diffusion coefficients, therefore a saw-tooth model, see chapter 17, was added to the TGA-specific diffusion model.

Franz diffusion cell method
Franz diffusion cell studies on neat chemical were performed on full or split thickness pig skin (from flank or back). The accumulated amount of chemical in the samples, taken from the Franz diffusion cell receptor fluid, is plotted over time in Figure 8-8, Figure 8-9 and Figure 8-10.
Figure 8-8 Accumulated amount of cyclohexanone (left axis), methanol (right axis) and 2-propanol (left axis) in receptor fluid, as measured by gas chromatography, from Franz diffusion cell experiments.

Figure 8-9 Accumulated amount of n-butanol, n-butyl acetate, n-hexane and toluene in receptor fluid, as measured by gas chromatography, from Franz diffusion cell experiments.
Figure 8-10 Accumulated amount of ethyl benzene, styrene and m-xylene in receptor fluid, as measured by gas chromatography, from Franz diffusion cell experiments.

The lag-times, steady-state fluxes, permeability coefficients, diffusion coefficients (Figure 8-11) and partition coefficients were calculated from the accumulated amount of chemical in the receptor fluid.

Figure 8-11 Diffusion coefficients (mean ± 95% CI) of neat chemical, obtained from Franz diffusion cell studies, plotted against their octanol:water partition coefficients.

Comparison of TGA and diffusion cell data
The diffusion coefficients obtained by TGA ($D_1$ and $D_2$) and Franz diffusion cell experiments ($D_F$) were compared, and a good correlation was seen between the faster of the two TGA obtained diffusion coefficients, $D_1$, and $D_F$, Figure 8-12 and Figure 8-13.
Figure 8-12 The faster of the two TGA obtained diffusion coefficients compared with the corresponding diffusion coefficient from the Franz cell experiments. The dotted line represents the line of unity.

Figure 8-13 Both TGA obtained diffusion coefficients (○ = D₁, ● = D₂) compared with the corresponding diffusion coefficient from the Franz cell experiments. The dotted line represents the line of unity.

As no morphological characteristics are assigned the compartments of the TGA-specific model, the obtained partition coefficients, $P_1$ and $P_2$, are arbitrary. However, the sum of the partition coefficients, $P_{TGA} = P_1 + P_2$, equals the skin:air partition coefficient of the epidermis. $P_{TGA}$ were compared with skin:air partition coefficients reported by other investigators using chemical vapor, and a good agreement was seen, Figure 8-14.
Figure 8-14 Comparison of TGA obtained skin:air partition coefficients, $P_{TGA} = P_1 + P_2$, with coefficients reported by other investigators (○=Scheuplein et al. 83, ●=Mattie et al. 59). The dotted line represents the line of unity.

An even better correlation was seen when comparing the TGA obtained permeability coefficients, $K_{p1}$ and $K_{p2}$, with those reported by other investigators using chemical vapor, Figure 8-15 and Figure 8-16.

Figure 8-15 Comparison of TGA obtained permeability coefficients, $K_{p1}$, with coefficients reported by other investigators 44, 49, 50, 63, 79, 83, 96. The dotted line represents the line of unity.
Figure 8-16 Comparison of TGA obtained permeability coefficients, $K_{p2}$, with coefficients reported by other investigators\textsuperscript{44, 49, 50, 63, 79, 83, 96}. The dotted line represents the line of unity.

8.4.2 Discussion

TGA method

The weight changes of the epidermal pieces were readily recorded for all of the studied chemicals.

An interesting finding was that the diffusion coefficients of the faster compartment were, for all studied chemicals (except n-butanol), 10-15 times higher than the coefficients of the second compartment. However, the partition coefficients of the second compartment were 2-3 times higher than those of the first compartment. In summary, the TGA-specific two compartment diffusion model has a fast compartment with low capacity and a slow compartment with high capacity.

In theory, as the diffusion coefficient is defined as the mobility of the molecules within the medium, it should be the same for any chemical concentration. However, this is not the case for the partition and permeability coefficients, as they vary with the studied chemical concentration. Therefore, the diffusion coefficients obtained by TGA and Franz cell experiments, are comparable. However, as the concentration of the chemical vapor is significantly lower than that of neat liquid, the partition and permeability coefficients obtained by the two methods are not comparable. Therefore, the TGA obtained partition and permeability coefficients must be compared to those obtained in more ideal situations.

The “true” volumes of the two compartments are not known as no morphological characteristics are assigned the two compartments, thus the obtained partition coefficients are arbitrary. However, the sum of the two coefficients, $P_{TGA}$, is the skin:air partition coefficient of the whole epidermis. A good agreement was found when comparing the TGA obtained partition coefficients, $P_{TGA}$, with coefficients obtained by other investigators studying chemical vapor.
When comparing the TGA obtained permeability coefficients with those reported by other investigators using chemical vapor, it was the “slower” compartment that showed the best agreement. This contradicts the previous finding where the diffusion coefficient of the “faster” compartment correlated to the coefficients obtained by Franz diffusion cell.

The TGA-specific two compartment diffusion model assumed two parallel diffusion pathways through the stratum corneum. Therefore, the resulting membrane resistance equals: \( \frac{1}{Z_{\text{tot}}} = \frac{1}{Z_1} + \frac{1}{Z_2} \) (equation 2-9) thus the corresponding total permeability coefficient \( K_{p,TGA} \) (cm/h) is calculated as \( K_{p,TGA} = K_{p1} + K_{p2} \). However, as the permeability coefficient of the first compartment, \( K_{p1} \), is significantly higher than that of the second compartment, \( K_{p2} \), the resulting \( K_{p,TGA} \) will approximately equal \( K_{p1} \).

Franz diffusion cell method
All of the studied chemicals were readily detected, for times > \( t_{lag} \), in the samples taken from the receptor fluid during Franz diffusion cell experiments.

When comparing the permeability coefficients obtained from the Franz diffusion cell studies, with those found in the literature, Table 1, a fairly good agreement was seen on data from human and pig skin for all chemicals except n-hexane. Thus, the very low permeability coefficients calculated from data found in Tsuruta 95 and Loden 57, could probably be explained by their use of physiological saline and human plasma as receptor medium, respectively. These mediums have a very low solubility for such a highly lipophilic substance as n-hexane thus the receptor medium will not act as a proper sink.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>( K_p ) (x10^4, cm/h)</th>
<th>Species</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>butanol, n-</td>
<td>2.7</td>
<td>pig</td>
<td>Rauma et al. 76</td>
</tr>
<tr>
<td></td>
<td>4.1-6.2^a</td>
<td>human</td>
<td>Boman et al. 12</td>
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<td></td>
<td>6.5^a</td>
<td>dog</td>
<td>DiVicenzo et al. 23</td>
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<td></td>
<td>10</td>
<td>human</td>
<td>Scheuplein et al. 84</td>
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<td>butyl acetate, n-</td>
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<td></td>
<td>5.3</td>
<td>pig</td>
<td>Rauma et al. 75</td>
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<tr>
<td>cyclohexanone</td>
<td>0.59</td>
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<td>Mraz et al. 65</td>
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<td>Rauma et al. 76</td>
</tr>
<tr>
<td>ethyl benzene</td>
<td>0.07^2</td>
<td>rat</td>
<td>Tsuruta 95</td>
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<td></td>
<td>5.3</td>
<td>pig</td>
<td>Rauma et al. 76</td>
</tr>
<tr>
<td></td>
<td>26^a</td>
<td>mouse</td>
<td>Susten et al. 93</td>
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<td></td>
<td>320</td>
<td>human</td>
<td>Dutkiewicz et al. 25</td>
</tr>
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<td>hexane, n-</td>
<td>0.00092^a</td>
<td>rat</td>
<td>Tsuruta 95</td>
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<td>0.01^a</td>
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<td>10</td>
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</tr>
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<td>Battice et al. 7</td>
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<td>Korinth et al. 53</td>
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<td></td>
<td>7.0</td>
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### Chemical $K_R$ (x10$^4$, cm/h) Species Study

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<th>Species</th>
<th>Study</th>
</tr>
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<td></td>
<td>99-170$^a$</td>
<td>human</td>
<td>Dutkiewicz et al. 27</td>
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<td>0.14</td>
<td>human</td>
<td>Wilkinson et al. 102</td>
</tr>
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<td></td>
<td>0.54$^a$</td>
<td>rat</td>
<td>Tsuruta 95</td>
</tr>
<tr>
<td></td>
<td>1$^a$</td>
<td>human</td>
<td>Ursin et al. 97</td>
</tr>
<tr>
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<td>1.6-2.4$^a$</td>
<td>human</td>
<td>Boman et al. 12</td>
</tr>
<tr>
<td></td>
<td>3.7$^a$</td>
<td>mini pig</td>
<td>Jacobs et al. 44</td>
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<td>pig</td>
<td>Rauma et al. 75</td>
</tr>
<tr>
<td></td>
<td>34$^a$</td>
<td>mouse</td>
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<td>210$^a$</td>
<td>human</td>
<td>Dutkiewicz et al. 26</td>
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<tr>
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<td>human</td>
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<td>xylene, m-</td>
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<td>human</td>
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<td>Rauma et al. 76</td>
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<td></td>
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<td>Engstrom et al. 29</td>
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<td>human</td>
<td>Riihimaki 78</td>
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</table>

$^a$The value is calculated from information found in article.
$^b$The study was performed on n-propanol.
$^c$The study was performed on 50% isopropanol in H$_2$O.

### Comparison of TGA and diffusion cell data

There are several advantages with the TGA method compared to Franz diffusion cells, thus small pieces of skin can be used (~0.5 cm$^2$), the method is not sensitive to holes and appendages in the skin so the demand for skin integrity is lower, the system can be fully automated and repeated exposures can be measured.

When comparing the diffusion coefficients obtained by the TGA and Franz diffusion cell methods, a good correlation was seen between the faster of the two TGA compartments ($D_1$) and the Franz diffusion cell coefficients ($D_F$). However, as expected from the large differences in chemical concentration, the permeability and partition coefficients were not comparable.

A very interesting correlation ($r=0.94$) was seen when comparing the logarithm of the ratios $D_1/D_F$ and $D_2/D_F$ against the logarithm of the octanol:water partition coefficients of the studied chemicals, Figure 8-17.

When adjusting the TGA obtained diffusion coefficients of the faster compartment ($D_1$) with the obtained power function ($\log(D_1/D_F)=0.596\cdot\log(P_{\text{octanol:water}})\cdot 0.272$) and comparing the adjusted values against the values obtained by Franz diffusion cell, a very nice correlation was seen, Figure 8-18.

Chemical concentration profiles in skin (for both TGA and Franz diffusion cell experiments) are presented in chapter 15.
Figure 8-17 The values $D_1/D_F (\circ)$ and $D_2/D_F (\bullet)$ are plotted against the corresponding octanol:water partition coefficients of the studied chemicals. The dotted lines represent the least square fit to each data set.

Figure 8-18 The faster of the two TGA obtained diffusion coefficients, adjusted by the obtained power function, compared with the corresponding diffusion coefficient from the Franz cell experiments. The dotted line represents the line of unity and the solid line represents the least square fit to the data set, respectively.
9 GENERAL DISCUSSION

Pig skin

Pig neonatal skin (piglets were stillborn or died within days after birth) was used in both the TGA and Franz diffusion cell experiments. Pig skin has been shown to be a good model for human skin \(^{90,91}\) and pig neonatal skin has also been shown to have similar properties as adult pig skin \(^{30,31}\) (although the opposite has also been shown \(^{68}\)). However, during the first days after birth, the skin most likely undergoes a huge adaptation from the aquatic in utero milieu to the outer environment. Therefore, skins from stillborn piglets were excluded (after the first study was performed).

Skins used in the TGA experiments were separated by means of trypsin. The average thickness of the separated skin pieces, 52 µm (n=30), is very similar to the reported thickness of pig epidermis, 66 µm \(^{14}\) and 60-85 µm \(^{43}\). Thus, it is assumed that the skin layers were separated at the epidermis/dermis junction. The same studies reported a stratum corneum thickness for pig skin of 26 µm and 17-28 µm, respectively.

Assessment of dermal absorption by TGA (Study I)

Several studies were found where TGA and other microbalance techniques where used in dermal absorption studies \(^{52,55,56,85}\). However, the use of TGA in dermal uptake studies of volatile chemicals has not previously been reported.

The TGA method is a non specific assay, as it is the total weight of the sample that is measured. Thus, the observed weight increase of a piece of skin exposed to chemical vapor could be due to; skin absorption of the chemical, a chemical induced increase (or decrease) in skin water content, and/or chemical adsorption on the skin surface. However, TGA experiments performed on an empty pan, showed no weight increase during exposure to chemical vapor (unpublished observations), indicating that adsorption is minimal.

When chemical vapor exposure ends, the chemical in the skin will evaporate into the surrounding air, resulting in a decrease in skin weight. It is possible to curve-fit the TGA-specific diffusion model to this “reversed” process, thereby using the TGA method to study the evaporation of chemical from skin.

Vapor generating system (Study II)

The vapor generating system contains seven gas wash bottles and two mass flow controllers (mfc), where the first mfc controls the airflow to the first two gas wash bottles (containing air and water), and the second mfc controls the airflow to the last five gas wash bottles (containing air, water, chemical 1, chemical 2 and chemical 3), respectively. If desired, the vapor generating system could be rearranged (by adding mass flow controllers) so several chemical vapors are generated simultaneously, thus facilitating TGA experiments studying dermal exposure to mixed chemical vapors.

An unused feature of the present vapor generating system (and the constructed computer program) is that the level of chemical vapor saturation in the airflow leading to the TGA instrument is adjustable (0-100%), thus facilitating TGA experiments studying dermal exposure to chemical vapors of different concentration.
Development of TGA-specific diffusion model (Study III)
The parallel, two compartment diffusion model, based on the derived TGA-specific solution to Fick’s second law, fits remarkably well to the TGA obtained weight curves. However, after studying stratum corneum morphology, it is apparent that the assumption of two independent parallel pathways is slightly erroneous. Thus, there is no direct connection between the corneocytes. Thus the molecules must diffuse through the inter-cellular matrix to reach the next level of corneocytes. Nevertheless, the obtained diffusion coefficients are similar to the ones obtained using the Franz cells, thus the model provides reasonable values.

The average time, \( t \), it takes for a molecule to diffuse the distance, \( x \), can be calculated by \( t=\frac{x^2}{2 \cdot D} \). Thus, for a piece of epidermis (thickness \( \approx 50 \mu m \)) exposed to e.g. methanol vapor (where \( D_1 \approx 2 \cdot 10^{-8} \text{ cm}^2/\text{s} \) and \( D_2 \approx 3 \cdot 10^{-9} \text{ cm}^2/\text{s} \)), the corresponding times to reach the bottom of the skin piece is approximately 10 and 70 minutes, respectively. Therefore, when fitting the TGA-specific model to the obtained skin weight curves, the faster of the two diffusion coefficients correlates to the initial shape of the weight curve. The observed noise on the TGA weight curves had a period of 10-15 minutes, which is close to the average diffusion times of the faster compartment (approximately 10 min). Therefore, a noise model was added to the TGA-specific diffusion model.

Validate TGA method against Franz cells (Study IV)
Differences between the TGA and Franz diffusion cell methods are;

**Skin hydration**
The skin in the TGA furnace is exposed to 45% RH whereas the skin mounted in the Franz diffusion cell is in direct contact with the receptor fluid, thus the epidermal sheet in the Franz cell has significantly higher water content. Blank and colleagues \(^9\) studied the dermal penetration of water in human skin (in vitro) at different hydration levels and found that an increase in skin hydration from 46 to 93%, approximately doubled the value of the diffusion coefficient. Bucks and colleagues \(^15\) studied the effect of occlusion on dermal penetration of steroids in human skin (in vivo) and found that the amount of absorbed progesterone (\( P_{\text{octanol/wat}} \approx 7500 \)) almost tripled (from 13 to 33%) when the exposure site was occluded (how the diffusion coefficient was affected is not mentioned in the article). Thus, increased skin hydration increases the dermal flux for both hydrophilic and lipophilic compounds.

**Skin preparation**
The skin used in TGA studies was separated by means of trypsin, whereas the skin in the Franz diffusion cell studies was split using dermatome. Both methods inflict damage to the skin, where trypsin makes the skin fragile (personal observation) and the dermatome introduces cuts. The affect on skin permeation is hard to quantify but the TGA method is most likely less sensitive to skin damage than the Franz cell method.

**Skin layers**
The skin in the Franz diffusion cell studies contains both epidermis and dermis, whereas in the TGA studies only epidermis is present. Dermis is usually a region of high water content, and in the Franz diffusion cell studies the fully hydrated dermis will probably act as a second diffusion barrier for lipophilic substances.

**Skin origin**
Due to practical reasons, the skins used in the TGA and Franz cell studies were from different sites of the pigs; ear and back/flank, respectively. Studies on water permeation on different body sites, show rather small differences \(^{87,88}\). Thus the diffusion coefficient for water on scalp skin is a factor of two larger than on skin from back \(^{88}\).
**Skin temperature**

The temperature of the skin in the TGA is 35°C, compared to 32°C for the skin in the Franz cells. It is well established that a higher temperature will result in a higher diffusivity\(^{10}\). Potts and colleagues\(^7\) studied the permeability of water in *stratum corneum* at several temperatures (20°C≤T≤90°C). When studying the published results (table 1 and figure 1 in the article), the increase in water permeability is constant for the temperature range 20-40°C. The water permeability as a function of the skin temperature, \(T\), can therefore be assumed by (from data in table 1):

\[
K_p = 0.86 \times 10^{-4} \cdot T - 1.5 \times 10^{-3} \text{ (cm/h) (20°C≤T≤40°C)}.
\]

From this equation, the water permeability at 32°C and 35°C is calculated to 1.3\(\times\)10\(^{-3}\) and 1.5\(\times\)10\(^{-3}\) cm/h, respectively. Thus, an increase in temperature by 3°C increases the water permeability 15%. One can assume that this applies to all studied chemicals.

**Chemical concentration**

Neat chemical was used in the Franz diffusion cell experiments, whereas chemical vapor was used in the TGA experiments. The use of neat chemical is problematic, as it affects the barrier properties, thus increasing the diffusivity. As the TGA situation is more ideal (approximately ten thousand-fold difference in concentration) less disturbance of skin barrier is expected in the TGA setup.

In summary, it has been observed (as seen in Figure 8-17) that hydrophilic compounds are more permeable in the Franz diffusion cells and lipophilic compounds are more permeable in the TGA. This observed correlation to the octanol:water partition coefficient of the studied chemical, is assigned the lower skin hydration in the TGA and the presence of a hydrated dermis in the skin used in the Franz cells, respectively. Although there is such extreme difference in skin hydration between the two methods, the obtained diffusion coefficients are remarkably similar.
10 CONCLUSIONS

Assessment of dermal absorption by TGA
- The weight change of pig epidermis, prior, during and after exposure to chemical vapor, was readily recorded using the TGA method.
- Differences in slope and height of the TGA obtained, skin weight curves, are contributed the differences in diffusion and skin:air partition coefficients of the studied chemicals, respectively.
- Only small skin samples are needed for the TGA experiments and skin integrity is not necessary.

Vapor generating system
- The computer-controlled vapor generating system is suitable for TGA exposure experiments, as the generated vapors are stable and reproducible and the dedicated computer program enables long-time runs and precise timing.
- The performance of the gas wash bottles is very good, as the air bubbling through chemical containing bottles reaches 90% saturation after approximately 5 s and thereafter achieving full saturation.

Development of TGA-specific diffusion model
- A TGA-specific solution to Fick’s second law of diffusion was derived, and from this solution a multi compartment parallel diffusion model was created.
- The diffusion model was curve-fitted to TGA obtained weight curves of four studied chemicals, and when applying the Akaike’s Information Criterion, it was found that a two compartment model was needed and sufficient.
- The first compartment of the model had a diffusion coefficient, \( D_1 \), which was significantly higher than that of the second compartment, \( D_2 \), whereas the second compartment had a higher partition coefficient, \( P_2 \), than the first compartment, \( P_1 \).

Validate TGA method against Franz cells
- The faster of the two TGA obtained diffusion coefficients, \( D_1 \), agreed well with the Franz diffusion cell obtained coefficients, \( D_F \).
- Good agreements were seen when comparing the TGA and Franz cell obtained partition and permeability coefficients with those reported by other investigators.
- An interesting finding was that the \( D_1/D_F \) ratios correlated nicely (\( r=0.94 \)) with the octanol:water partition coefficients of the studied chemicals. The ratio was above 1 for lipophilic and below 1 for hydrophilic substances, respectively.

In conclusion, it appears that good estimates of the diffusion and skin:air partition coefficients for volatile chemicals can be obtained by using the TGA technique and analyzing the obtained weight curves with a two compartment model.
11 SAMMANFATTNING

Huden är kroppens tyngsta organ (~7 kg) och står för ca 15% av en vuxen persons kroppsvikt. Den täcker hela kroppen (~1.8 m²) och har till uppgift att skydda mot uttorkning samt mot biologiska, kemiska och mekaniska faror. Hudens tjocklek är ungefär 2-4 mm och är tunnast på ögonlocken och tjockast på handflator och fotsulor.

Huden består av tre urskiljbara lager (från insidan och ut); underhuden, läderhuden och överhuden där de översta 10-30 cellagren i överhuden kallas stratum corneum. Detta skikt är ca 10 µm tjockt och består framförallt av tillplattade, tätt packade döda hudceller (korneocyter, ~90% av volymen) omgärda av fett. Denna konstruktion gör att molekyler som vill penetrera stratum corneum måste ta en lång och vindlig väg genom den fettrika strukturen, vilket gör att den effektiva vägen genom huden är 10-100 ggr längre än tjockleken på stratum corneum. Molekylernas penetrationshastighet genom huden bestäms av flera faktorer, t.ex. deras storlek (vikt) och löslighet.

Det råder stor brist på hudupptagsdata, då endast ca 100 av de 100 000 förekommande kemikalierna har testats. Många av dessa försök har utförts på mus, rätta och marsvin så relevansen för människa är osäker. Dessutom är spridningen ibland mycket stor för rapporterade data (för ämnet fenol är den ungefär 10 000 000). Detta gör att det vetenskapliga underlaget för t.ex. riskbedömningar och hygieniska gränsvärden försvagas.

Strukturen hos apans och grisens hud är mycket lik människohud och kan med fördel användas i hudupptagsstudier. Hud från bepälsade djur, som t.ex. mus, rätta och marsvin visar oftast ett mycket högre upptag av kemikalier än för människohud.

Flera tillgängliga metoder finns för att mäta hudupptag av kemikalier.
- In vivo metoder utförs på levande djur eller människor. Huden exponeras för en känd mängd kemikalie och efter att ha diffunderat genom huden når kemikalien blodet. Efter analys av blodprov kan penetrationen beräknas. Försöken ger relevant information men de största nackdelarna med in vivo metoder är att de ur etisk synpunkt kan vara svåra att genomföra och att de är kostsamma.
- In vitro metoder utförs på avlägsnad hud. Huden spänns då fast i en hållare (s.k. diffusionscell) där hudens undersida är i direkt kontakt med ett receptormedium. En känd mängd kemikalie placeras på hudens ovansida och efter att ha diffunderat genom huden når kemikalien receptormediet. Efter analys av receptormediet kan penetrationen beräknas. Diffusionscellerna ger tämligen relevant information och de största nackdelen är att metoden inte är fullt standardiserad.

Behovet finns alltså för en ny, standardiserad in vitro metod. Syftet med denna avhandling var att utveckla och testa den termogravimetriska analysmetoden (TGA) för att mäta hudupptaget av kemikalieånga. TGA är i princip en våg med hög känslighet där provet är placerat i en ugn (20-1000°C) och provets vikt mäts tre ganger per sekund. Då huden exponeras för kemikalieånga kommer molekylerna att diffundera in i huden och den totala vikten (hud + kemikalie) kommer då att öka.
Väga hudupptag av kemikalieånga med TGA
Runda bitar (ø8 mm) av överhud från gris (baksida av öra från självdöda spädgrisar) placerades i TGA vågen och temperaturen hölls konstant på 35°C.


Resultaten visade att man med god noggrannhet kunde mäta hudens viktföreändring då den exponerades för kemikalieånga. Då typiska viktkurvor från de fyra kemikalierna jämfördes sågs tydliga skillnader i stigtid och viktökning, kopplat till de studerade kemikaliernas diffusionsparametrar.

Datorstyrt ånggenereringssystem
Ett datorstyrt system för generering av kemikalieånga byggdes med hjälp av flödesregulatorer, magnetventiler och gastvättflaskor. Genom att flöda luft genom kemikalien (som vätska i gastvättflaskorna) kunde genereringen av kemikalieånga hållas på en hög nivå. Gastvättflaskorna placerades i ett tempererat vattenbad då genereringen av kemikalieånga är känslig för temperaturförändringar. Med hjälp av ett specialskrivet datorprogram kunde tidskrävande försök automatiseras.

TGA specifik matematisk diffusionsmodell

Validera TGA metoden med diffusionscellstudier
Hudupptagsstudier på grishud utfördes med både TGA och Franz diffusionsceller på 10 kemikalier (n-butanol, n-butylacetat, cyklohexanon, etylbensen, n-hexan, metanol, 2–propanol, styren, toluen och m-xilen). Kemikalie-specifica diffusionsparametrar (diffusionskoefficienten) beräknades för alla ämnena och jämfördes mellan metoderna, där det visade sig att den snabbare av de två TGA parametrarna gav bäst överensstämmelse med parametrarna från Franz diffusionscellstudierna.

Sammanfattningsvis, så är fördelarna med att använda TGA metoden att endast små hudbitar behövs, hudbitar med hål i kan användas, upprepade exponeringar för kemikalieånga kan utföras samt att kemikaliernas diffusion i huden kan studeras på ett nytt sätt.

Denna avhandling har visat att det går att väga hudupptaget av kemikalieångor med stor precision med TGA metoden samt att de diffusionsparametrar man beräknar (med hjälp av den matematiska modellen) är rimliga.
12 ACKNOWLEDGEMENTS

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13 REFERENCES


14 APPENDIX A – TGA-SPECIFIC SOLUTION TO FICK’S 2ND LAW

The TGA-specific solution to Fick’s second law:

$$\frac{\partial C_{\text{skin}}(x,t)}{\partial t} = D \cdot \frac{\partial^2 C_{\text{skin}}(x,t)}{\partial x^2} \quad 0 \leq x \leq h; \ t > 0$$

(A-1)

is obtained by using ordinary differential equation (ODE) solving techniques, and the method of “separation of variables” (see e.g. Boyce et al. 13).

The first step of solving $C_{\text{skin}}(x,t)$ is to assume that the function can be separated into two components:

$$C_{\text{skin}}(x,t) = C_{ss}(x) + C_{\text{dyn}}(x,t)$$

(A-2)

where $C_{ss}(x)$ is the steady-state concentration distribution (as our skin model assumes a homogenous piece this variable is actually a constant, $C_{ss}$) and $C_{\text{dyn}}(x,t)$ is the dynamic concentration, i.e. time dependent, distribution. The dynamic component is further separated into a depth-dependent and a time-dependent part, yielding:

$$C_{\text{dyn}}(x,t) = C_{\text{dyn}}(x) \cdot C_{\text{dyn}}(t)$$

(A-3)

Combining equations A-1, A-2 and A-3 and defining $C_{ss}$ a constant, yields:

$$\frac{\partial C_{\text{dyn}}(x,t)}{\partial t} = D \frac{\partial^2 C_{\text{dyn}}(x,t)}{\partial x^2} \Rightarrow C_{\text{dyn}}(x) \cdot C'_{\text{dyn}}(t) = D \cdot C_{\text{dyn}}(t) \cdot C''_{\text{dyn}}(x)$$

(A-4)

After rearranging equation A-4:

$$\frac{C''_{\text{dyn}}(x)}{C_{\text{dyn}}(x)} = \frac{1}{D} \frac{C'_{\text{dyn}}(t)}{C_{\text{dyn}}(t)} = -\lambda^2$$

(A-5)

where the left side only depends on skin depth, $x$, and the right side only depends on time, $t$. This relationship must be equal for all depths ($0 \leq x \leq h$) and all times ($t > 0$) and therefore it must be a depth and time independent constant, here called $-\lambda^2$, where the minus sign is needed to assure real solutions. Equation A-5 will now result in two new differential equations:

$$C''_{\text{dyn}}(x) + \lambda^2 \cdot C_{\text{dyn}}(x) = 0$$

(A-6)

$$C'_{\text{dyn}}(t) + D \cdot \lambda^2 \cdot C_{\text{dyn}}(t) = 0$$

(A-7)

The components $C_{\text{dyn}}(x)$ and $C_{\text{dyn}}(t)$ can now be identified, through standard ODE solutions, as:

$$C_{\text{dyn}}(x) = k_1 \cdot \sin(\lambda \cdot x) + k_2 \cdot \cos(\lambda \cdot x)$$

(A-8)

$$C_{\text{dyn}}(t) = k_3 \cdot e^{-D \cdot \lambda^2 \cdot t}$$

(A-9)

where the constants $k_1$, $k_2$, $k_3$ and $\lambda$ will later be identified using the specific boundary and initial conditions of the TGA setup, equations 7-4, 7-5, 7-6 and 7-7. The dynamic component is now rewritten by combing equations A-3, A-8 and A-9, as:

$$C_{\text{dy}(x,t)} = C_{\text{dy}}(x) \cdot C_{\text{dy}}(t) = (k_1 \cdot \sin(\lambda \cdot x) + k_2 \cdot \cos(\lambda \cdot x)) \cdot k_3 \cdot e^{-D \cdot \lambda^2 \cdot t}$$

(A-10)
Combining equations A-2 and A-10 gives:

\[ C_{\text{skin}}(x,t) = C_{ss} + \left( k_1 \cdot \sin(\lambda \cdot x) + k_2 \cdot \cos(\lambda \cdot x) \right) \cdot k_3 \cdot e^{-D \cdot \lambda^2 \cdot t} \]  

(A-11)

which is a general solution to Fick’s second law for a homogenous piece of skin, i.e. constant diffusion coefficient \( D \), exposed to a constant vapor concentration when assuming only transdermal (vertical) diffusion.

A TGA-specific solution to equation A-11 is obtained by applying the TGA-specific boundary conditions. This yields:

\[ C_{\text{skin}}(x,t) = C_{ss} + c_n e^{\frac{(2n+1)^2 \pi^2 x^2}{4h^2}} \sin \left( \frac{(2n+1)\pi x}{2h} \right) \]  

\( n = 0,1,2,... \)  

(A-12)

where \( \lambda = \frac{(2n+1)\pi}{2h} \) and \( c_n = k_1 \cdot k_3 \). Based on the principle of superposition and that the differential equation and boundary conditions are linear and homogenous, equation A-12 may be expressed as a serial expansion (see e.g. Boyce et al.):

\[ C_{\text{skin}}(x,t) = C_{ss} + \sum_{n=0}^{\infty} c_n e^{\frac{(2n+1)^2 \pi^2 x^2}{4h^2}} \sin \left( \frac{(2n+1)\pi x}{2h} \right) \]  

(A-13)

As \( C_{ss} \) is defined as the concentration within the homogenous skin piece at steady-state and the chemical vapor concentration exposing the skin is constant, therefore:

\[ C_{ss} = P_{\text{skin,air}} \cdot C_{TGA} \]  

(A-14)

When applying the Fourier theorem (see e.g. Boyce et al.), the initial conditions and equations A-13 and A-14, make it possible to identify the coefficient \( c_n \) as:

\[ c_n = -4 \cdot \frac{P_{\text{skin,air}} \cdot C_{TGA}}{(2n+1)\pi} \int_0^h \left( C_0(x) - P_{\text{skin,air}} \cdot C_{TGA} \right) \sin \left( \frac{(2n+1)\pi x}{2h} \right) dx \]  

(A-15)

Where \( C_{TGA} \) is the constant chemical concentration in the TGA furnace (g/cm³), \( C_0(x) \) is the chemical concentration in the skin prior exposure (g/cm³), i.e. initial condition, for the TGA specific situation \( C_0(x)=0 \). By combining equations A-13, A-14 and A-15, the concentration profile of the chemical in the exposed skin, can now be expressed as:

\[ C_{\text{skin}}(x,t) = P_{\text{skin,air}} \cdot C_{TGA} \cdot \left( 1 - 4 \sum_{n=0}^{\infty} \frac{1}{(2n+1)} e^{\frac{(2n+1)^2 \pi^2 x^2}{4h^2}} \sin \left( \frac{(2n+1)\pi x}{2h} \right) \right) \]  

(A-16)

As the TGA instrument records the total weight of the exposed skin piece, the total skin weight equation is therefore the sum of the initial skin weight and the weight of the absorbed/desorbed chemical in the skin, i.e.:

\[ W_{\text{skin}}(t) = W_{\text{base}} + A \cdot \int_0^h C_{\text{skin}}(x,t) dx \]  

(A-17)

where \( W_{\text{skin}}(t) \) is the total weight of the skin (mg), \( W_{\text{base}} \) is the constant weight of the skin (mg) prior to exposure to chemical vapor and \( A \) is the total skin area (cm²) exposed to chemical vapor. The full skin weight function for the TGA-specific conditions is obtained by combining equations A-16 and A-17:

\[ W_{\text{skin}}(t) = W_{\text{base}} + P_{\text{skin,air}} \cdot C_{TGA} \cdot A \cdot h \cdot \left( 1 - 8 \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} e^{\frac{(2n+1)^2 \pi^2 x^2}{4h^2}} \right) \]  

(A-18)
APPENDIX B – CONCENTRATION PROFILES OF CHEMICAL IN SKIN

Skin mounted in Franz diffusion cells

In the Franz diffusion cell, the upper part of the skin is exposed to a known concentration of chemical, $C_{\text{donor}}$, whereas the bottom part of the skin lays on the receptor medium. As the volume of the receptor compartment is very large, the chemical concentration, $C_{\text{receptor}}$, will be very low during the whole experiment, thus it is considered to be a sink.

The chemical concentration in the skin over time can be described (see e.g. Crank\textsuperscript{22}) as:

\[
C_{\text{skin}}(x,t) = P_{\text{skin:vehicle}} \cdot C_{\text{donor}} \left[ 1 - \frac{x}{h} - \sum_{n=1}^{\infty} \frac{1}{n} \sin \left( \frac{n\pi x}{h} \right) \cdot e^{-\frac{n^2 \pi^2 D t}{h^2}} \right]
\]  

(B-1)

Figure B-1 displays the chemical concentration profile at several time points.

Figure B-1 Plotted concentration profile of chemical in skin, $C_{\text{skin}}(x,t)$, equation B-1, at different time-points, $D t/h^2$. Data for e.g. methanol, $D=6.4 \times 10^{-8}$ cm$^2$/s and $h \approx 300$ µm, suggests that $D t/h^2=1.0$ corresponds to $t \approx 4$ h.
**Skin placed in TGA-furnace**

In the TGA, the upper part of the skin is exposed to a known concentration of chemical vapor, \( C_{TGA} \), whereas the bottom of the skin is in close contact with the platinum pan so no net flux will occur over that surface.

The chemical concentration in the skin over time can be described by (see chapter 8.3):

\[
C_{\text{skin}}(x,t) = P_{\text{skin,air}} \cdot C_{TGA} \left( 1 - \frac{4}{\pi} \sum_{n=1}^{\infty} \frac{1}{2n-1} \sin \left( \frac{(2n-1)\pi x}{2h} \right) e^{-\frac{D(2n-1)^2\pi^2}{4h^2}t} \right) \tag{B-2}
\]

Figure B-2 displays the chemical concentration profile at several time points for the one compartment model.

![Figure B-2 Plotted concentration profile, \( C_{\text{skin}}(x,t) \), of chemical in skin, equation B-2, at different time points, \( Dt/h^2 \). Data for e.g. methanol, \( D=2.4 \times 10^{-8} \text{ cm}^2/\text{s} \) and \( h=70 \mu\text{m} \), suggests that \( Dt/h^2=1.0 \) corresponds to \( t=0.5 \text{ h} \).](image)

Figure B-3 displays the chemical concentration profile at several time points for the two compartment model when assuming that the first compartment \((D_1, P_1)\) is fast with low capacity and the second \((D_2, P_2)\) is slow with high capacity \((D_1=10\cdot D_2 \) and \( P_1=0.5 \cdot P_2 \).
Figure B-3 Plotted concentration profile, $C_{\text{skin}}(x,t)$, of chemical in skin for the TGA-specific two compartment diffusion model, variant of equation B-2, at different time points, $Dt/h^2$. It is assumed that the first compartment was fast having low capacity and the second was sloe having high capacity, thus $D_1 = 10 \cdot D_2$ and $P_1 = 0.5 \cdot P_2$. Therefore, the time points 0.1 and 1.0 corresponds to the times when the first and second compartments are full, respectively. Data for e.g. methanol, $D_1 = 2.4 \cdot 10^{-8}$ cm$^2$/s, $D_2 = 2.7 \cdot 10^{-9}$ cm$^2$/s and $h \approx 70$ µm, suggests that $Dt/h^2 = 1.0$ corresponds to $t \approx 5$ h and $Dt/h^2 = 0.1$ corresponds to $t \approx 0.5$ h, respectively.
16 APPENDIX C – GAS WASH BOTTLE PERFORMANCE

The vapor generating performance of a gas wash bottle was tested using a photo ionization detector (PID) connected to a diluting system, as described in chapter 7.5.3. Cyclohexanone was chosen as test substance as it has a low vapor pressure, 0.594 kPa corresponding to 5868 ppm at 25°C. Several dilutions were performed and the concentration of cyclohexanone was measured every 15 seconds (all measured concentrations within the linear range of the PID), and the corresponding vapor concentrations were calculated, Table C-1.

Table C-1 Concentrations of cyclohexanone as measured using PID.

<table>
<thead>
<tr>
<th>Total flow (l/min)</th>
<th>Dilution (1)</th>
<th>PID measured conc. (ppm)</th>
<th>Converted conc. (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00</td>
<td>45</td>
<td>130</td>
<td>5710</td>
</tr>
<tr>
<td>2.05</td>
<td>57</td>
<td>102</td>
<td>5760</td>
</tr>
<tr>
<td>3.00</td>
<td>68</td>
<td>87</td>
<td>5900</td>
</tr>
<tr>
<td>3.05</td>
<td>79</td>
<td>75</td>
<td>5920</td>
</tr>
<tr>
<td>4.00</td>
<td>90</td>
<td>67</td>
<td>5990</td>
</tr>
<tr>
<td>4.05</td>
<td>101</td>
<td>59</td>
<td>5960</td>
</tr>
<tr>
<td>5.00</td>
<td>110</td>
<td>53</td>
<td>5920</td>
</tr>
</tbody>
</table>

The average concentration was 5881 ± 103 ppm (mean ± sd), corresponding to 100.2 ± 1.7% of the calculated saturated concentration.

A mono-exponential equation was fitted to the PID results, Figure C-1, to estimate the time course of the detector response, as:

\[ C = C_{SS} \left(1 - e^{-kt}\right) \]  

(C-1)

where \( C \) is the PID response (ppm), \( C_{SS} \) is the steady-state concentration (ppm) and \( k \) is the rate constant (s\(^{-1}\)). The average rate constant was 0.44 ± 0.08 s\(^{-1}\), and the corresponding \( t_{50\%} \) and \( t_{90\%} \) were 1.6 ± 0.3 and 5.4 ± 0.8 s, respectively.

![Figure C-1 15 s average (o) time course of the PID response to measured cyclohexanone concentration in air that was bubbled through a cyclohexanone containing gas wash bottle. To reach concentrations where the PID had a linear response, a diluting system was used.](image)
APPENDIX D – MODELING THE TGA NOISE

During the TGA measurements, a small and cyclic, saw-tooth shaped noise was observed on the recorded weight curve, having a period of approximately 10-15 minutes, as mentioned in chapter 8.2.2.

As the parameters of the TGA-specific diffusion model are estimated by curve-fitting the model to TGA obtained weight curves and the value of the faster diffusion coefficient is highly affected by the initial shape of the weight curve, the cyclic noise must be included into the diffusion model. Therefore, a cyclic, saw-toothed noise model was created in Berkeley Madonna.

![Figure D-1 The cyclic, saw-toothed noise was modeled in Berkeley Madonna.](image)

The saw-toothed noise model, Figure D-1 and Figure D-2, has three parameters, where $W_{\text{noise}}$ is the weight increase (mg) (approximately 0.3 µg), $T_{\text{period}}$ is the repetition time (min) (approximately 10-15 min) and $T_{\text{up}}$ is the time to reach maximum weight (min) (approximately 20% of $T_{\text{period}}$).

![Figure D-2 A TGA-specific diffusion model fitted to the recorded weight of a piece of epidermis (background black uneven line) exposed to known concentration of cyclohexanone. The model is fitted without the noise model (dashed line) and with the noise model (thick line).](image)