Forensic taphonomy in an indoor setting

Implications for estimation of the post-mortem interval

ANN-SOFIE CECILIASON
Abstract

The overall aim of this thesis was to determine if and how taphonomic data can be used to expand our knowledge concerning the decompositional process in an indoor setting, as well as adapting scoring-based methods for quantification of human decomposition, to increase the precision of post-mortem interval (PMI) estimates.

In the first paper, the established methods of Total Body Score (TBS) and Accumulated Degree-Days (ADD) were investigated in an indoor setting, with results indicating a fairly low precision. The PMI was often underestimated in cases with desiccation and overestimated in cases with presence of insect activity. This suggests that the TBS method needs to be slightly modified to better reflect the indoor decompositional process.

In the second paper, a novel method for PMI estimation was developed using histological assessment of decompositional changes in the human liver. The scoring-based method created, the Hepatic Decomposition Score, was a statistically robust way to quantify the degree of decomposition, with the potential to improve the precision of PMI estimates.

In the third paper, the indoor decomposition process was further investigated regarding microbial neoformation of volatiles in relation to the degree of decomposition and the PMI. A higher decomposition degree was observed in cases with neoformation (i.e., presence of N-propanol and/or 1-butanol in femoral vein blood) than in cases without signs of neoformation. Microbial neoformation may be an indicator of decomposition rate, which may make it possible to improve the precision of PMI estimates based on the TBS/ADD method.

In the fourth paper, a novel constructed Bayesian framework allowed a qualified estimate of PMI based on observed taphonomic findings. This framework provided a unique possibility to report results, express the uncertainties in assumptions and calculations, as well as to evaluate competing hypotheses regarding PMI periods or time of death.

Taken as a whole, the results indicate that using taphonomic data derived from an indoor setting could improve scoring-based methods, as well as highlighting benefits of incorporating such data into a Bayesian framework for interpretational purposes and for reporting PMI estimates.

Keywords: Forensic taphonomy, Indoor setting, Post-mortem interval estimation, Hepatic decomposition score, Total body score, microbial neoformation

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Dedicated to you, who are drawn to read it
This thesis is based on the following papers:


The papers are referred to in the text by their Roman numerals.
# Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>11</td>
</tr>
<tr>
<td>Post-mortem interval estimation</td>
<td>12</td>
</tr>
<tr>
<td>Forensic taphonomy</td>
<td>12</td>
</tr>
<tr>
<td>The decompositional processes</td>
<td>13</td>
</tr>
<tr>
<td>Factors affecting the rate and pattern of decomposition</td>
<td>14</td>
</tr>
<tr>
<td>Human decomposition in an indoor setting</td>
<td>15</td>
</tr>
<tr>
<td>Temperature and Accumulated Degree-Days</td>
<td>17</td>
</tr>
<tr>
<td>The indoor climate</td>
<td>17</td>
</tr>
<tr>
<td>Decomposition during morgue storage</td>
<td>17</td>
</tr>
<tr>
<td>Quantifying the decompositional process</td>
<td>18</td>
</tr>
<tr>
<td>Total Body Score method</td>
<td>19</td>
</tr>
<tr>
<td>Assessing taphonomic data and reporting PMI estimates</td>
<td>23</td>
</tr>
<tr>
<td>Aim of thesis</td>
<td>25</td>
</tr>
<tr>
<td>Aim of each study</td>
<td>25</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>26</td>
</tr>
<tr>
<td>Selection of cases</td>
<td>26</td>
</tr>
<tr>
<td>General methodology and study design</td>
<td>27</td>
</tr>
<tr>
<td>Statistical analyses</td>
<td>29</td>
</tr>
<tr>
<td>Ethical considerations</td>
<td>30</td>
</tr>
<tr>
<td>Results</td>
<td>31</td>
</tr>
<tr>
<td>Paper I</td>
<td>31</td>
</tr>
<tr>
<td>Indoor decomposition</td>
<td>31</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>32</td>
</tr>
<tr>
<td>Paper II</td>
<td>35</td>
</tr>
<tr>
<td>The Hepatic Decomposition Score</td>
<td>35</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>37</td>
</tr>
<tr>
<td>Paper III</td>
<td>38</td>
</tr>
<tr>
<td>Relationship between detected volatiles and TBS or PMI</td>
<td>39</td>
</tr>
<tr>
<td>The TBS/ADD method</td>
<td>40</td>
</tr>
<tr>
<td>Rate-modified $\log_{10}$ADD model</td>
<td>40</td>
</tr>
<tr>
<td>Paper IV</td>
<td>41</td>
</tr>
<tr>
<td>Relationship between ADD and partial body scores</td>
<td>41</td>
</tr>
<tr>
<td>Choosing a prior</td>
<td>41</td>
</tr>
<tr>
<td>Likelihood ratios for competing hypotheses</td>
<td>42</td>
</tr>
</tbody>
</table>
Abbreviations

ADD – Accumulated day-degrees
AM – Ante-mortem
AT – Accumulated temperature
BMI – Body mass index
EM – Expectation maximisation
HDS – Hepatic decomposition score
ICC – Intra-class correlation
log\textsubscript{10} – Common logarithm (base 10)
LR – Likelihood ratio
ML – Maximum likelihood algorithm
PBSH – Partial body score head
PBSL – Partial body score limbs
PBST – Partial body score trunk
PMI – Post-mortem interval
SD – Standard deviation
SEM – Standard error of measurement
TBS – Total body score
Introduction

A central task of the forensic investigation is to determine a plausible cause and manner of death, as well as time of death. The post-mortem interval (PMI) is the time elapsed from the time of death to when the body is discovered. A correct estimation of a PMI can therefore give an estimated time of death, which may be crucial for example in a suspected murder, where conflicting information has been provided by witnesses and potential offenders. A reliable PMI estimate can also be useful in natural deaths, for example, as an aid in identification of the deceased, to help relatives in their grief processes (e.g., reliable date of death), or in cases of inheritance and insurance disputes. The assessment of the occurrence and concentration of various drugs is in many ways affected by PMI; thus, a proper estimate may be helpful in assessing a probable poisoning or an overdose. In Sweden, PMI estimation is almost exclusively carried out upon suspicion of homicide, but it can also be useful to assess PMI in other types of cases/deaths to increase the quality of the forensic investigation.

Knowledge of when different types of decompositional changes occur and of their subsequent effects could significantly increase the accuracy and precision of PMI estimation. Human remains can stay undiscovered for prolonged periods of time. Advanced decomposition may affect the possibility to correctly determine the cause and manner of death due to difficulties in interpretation of injuries and pathological changes. Understanding of the decompositional processes that a dead body will invariably undergo, and the different factors affecting the decomposition, is also of great importance to forensic investigations.

Approximately 25% of all the forensic autopsy cases/year in Sweden exhibit decompositional changes to a various extent. There are a total of around 1,500 cases, where the majority of cases are discovered in an indoor setting. This specific environment is without exposure to wind, rain, sun, or large temperature fluctuations. Limitations in insect access and animal scavengers is also prominent. Other factors, e.g., position of the body, clothing or coverings, body size and weight, pre-existing diseases and pathological lesions, and trauma/injuries may therefore have a large impact on the indoor decompositional process. However, the extent of this impact is essentially unknown, since research in this specific setting is rather limited.
Post-mortem interval estimation

There is currently a vast number of methods for determination of PMI (with physical, chemical, biological, and entomological approaches). For example, estimation of PMI could be based on post-mortem changes such as rigor mortis, livor mortis, and algor mortis [Henssge et al. 2002, Rodrigo 2016], muscle excitability [Henssge et al. 2002, Elmas et al. 2001], gastric content emptying [Henssge et al. 2002], chemical composition of the vitreous humour [Zilg et al. 2015, Rognum et al. 2016], biochemical markers in blood, e.g., volatile fatty acids, amino acids and metabolites [Vass et al. 2002, Swann et al. 2010, Viinamaki et al. 2011], immunohistochemical staining for thyroglobulin [Wehner et al. 2000], microbial succession [Metcalf et al. 2016, Pechal et al. 2014], gene expression [Kimura et al. 2011, Javan et al. 2015], and RNA [Lv et al. 2016, Scrivano et al. 2019] or DNA degradation [Perry et al. 1988, Tozzo et al. 2020]. Depending on the circumstances, these methods can yield results in a narrow or wide interval. Several techniques are limited to a particular stage of the PMI and a specific type of observation. Henssge and Madea [2004] indicate that a reliable determination of PMI is only possible for up to 72 hours. However, under certain circumstances, forensic entomology may specify a PMI of up to several months [Campobasso et al. 2001, Amendt et al. 2007].

Sledzik [1998] stated that one way to consider decomposition is as a linear progression. Different scientific methods are employed at different points along this line, to determine how much time has elapsed since death. However, decomposition rates are notably variable due to anatomical variation between persons [Knight and Saukko 2004] and environmental conditions [Sledzik, 1998, Knight and Saukko 2004], resulting in difficulties in ascribing a precise PMI value. Often, only an estimate can be presented, if that.

Henssge and Madea [2007] stated that the method for PMI estimation must include several specific criteria to gain practical relevance; quantitative measurement, mathematical description, quantification of influencing factors and precision of the method is presented and validation using an independent material.

Forensic taphonomy

The term taphonomy, meaning the law of burial, was first introduced to palaeontology by Efremov [1940] and derives from the Greek words *taphos* meaning burial and *nomos* meaning law [Nawrocki 1996]. Today, taphonomy intersects with several different academic fields, such as archaeology, entomology, botany and palynology, mycology, forensic science, anthropology, and forensic pathology.
In their work, Haglund and Sorg [1997] presented a comprehensive definition of forensic taphonomy:

“Forensic taphonomy refers to the use of taphonomic models, approaches, and analyses in forensic contexts to estimate the time since death, reconstruct the circumstances before and after deposition, and discriminate the products of human behavior from those created by the earth’s biological, physical, chemical, and geological subsystems.”

As early as in 13th century China, the human decompositional process was described in detail by Sung Tz’u in his forensic medicine book, *The Washing Away of Wrongs* [Sung Tz’u 1186–1249]. The interest in knowing what happens to the human body after death is by no means new.

The decompositional processes

Post-mortem changes start to develop immediately after death, as the decrease of body temperature, lividity and rigidity are followed by autolysis (i.e., digestion of tissue by cellular enzymes) and putrefaction (i.e., enzymatic activity of fungi and bacteria). The early decomposition is characterised by abdominal discoulouration, skin slippage (i.e., loss of epidermis), and hair loss, followed by bloating of the face and abdomen, and purging of decompositional fluids from facial orifices [Pinheiro 2006]. Putrefactive bacteria produce gasses resulting in bloating of the body as well as discoulouration of the skin (i.e., hydrogen sulphide reacting with haemoglobin forming sulfhaemoglobin) [Pinheiro 2006, Goff 2010]. The accumulated gasses also promote transport of sulfhaemoglobin via the circulatory and lymphatic system, resulting in the characteristic marbled appearance of the body [Pinheiro 2006]. Several putrefactive bacteria can produce ethanol via fermentation (i.e., a chemical process by which molecules are degraded anaerobically), probably utilising glucose and other carbohydrates, as well as amino acids and lipids [Corry 1978, Bogusz et al. 1970]. During fermentation, other volatiles may also be produced post-mortem, such as acetaldehyde, acetone, 1-butanol, N-propanol, and isopropanol [Corry 1978, Boumba et al. 2008]. The neoformation (i.e., bacterial post-mortem production) of ethanol in a decomposed body is often below 0.70 mg/ml [Gilliland and Bost 1993], although amounts of 1.50 mg/ml to 2.20 mg/ml have been documented [Gilliland and Bost 1993, Zumwalt et al. 1982]. These levels of ethanol are of forensic interest and it is therefore of relevance to exclude with certainty if ethanol can be of ante-mortem origin by active intake. The occurrence of neoformation of ethanol in decomposed human bodies has been reported to be around 18 to 22% [Zumwalt et al. 1983, Gilliland and Bost 1993].
Abdominal gasses are later released resulting in a deflated-looking abdomen, and within the same time period the green discolouration begins to progressively turn blacker [Pinheiro 2006, Goff 2010]. Volatile organic compounds (VOCs) are by-products of the decompositional process and associated with the odour from decomposing human remains. The production of VOCs can be linked to specific bacterial species [Cernosek et al. 2020] and could possibly also be used as an indicator for the PMI [Paczkowski et al. 2015].

During the active decay stage, the greatest mass loss occurs due to liquefaction of tissues, disintegration, and purging of decompositional fluids into surrounding environment [Carter and Tibbett 2008]. If insects have access to the dead body, maggots feeding are responsible for a large part of the mass loss [Bass 1997, Simmons et al. 2010]. Gradually, bone becomes exposed, potentially with cartilage, hair, and desiccated tissue left, and the remains may progress to full skeletonization [Teo et al. 2014, Pinheiro 2006, Goff 2010].

The body and its internal organs do not decompose in the same way or at the same speed. The ileocecal area hosts the largest amounts of bacteria, which after death spread to the liver and spleen, and further to the heart and brain, depending on the cause of death [Javan et al. 2019]. This post-mortem bacterial activity is suggested to cause a domino effect that can drive the order of human decomposition [Javan et al. 2019]. The organs exhibiting early signs of decomposition include the gastrointestinal tract, pancreas, and liver. The heart and blood vessels may take a longer time to decompose [Javan et al. 2019]. The most resistant organ is the uterus, while tissues like the tendons and bones also remain intact longer [Javan et al. 2019]. The post-mortem changes in soft tissues and internal organs can be used to give an estimate of the PMI until skeletonization is achieved. However, the rate of decomposition can be considerably altered by both internal and external factors such as temperature, insect activity, animal scavenging, trauma, cause of death, environmental conditions, clothing, and body size [Rodriguez and Bass 1985, Micoczi 1986, Vass et al. 1992, Komar 1998, Campobasso et al. 2001]. Bone decomposition is caused by weathering due to environmental conditions and erosion depending on soil conditions [Wilson-Taylor 2013], usually associated with outdoor decomposition. Cases with canine scavenging in an indoor environment have been described [Steadman and Worne 2007] and could be a possible factor in destruction of bone.

Factors affecting the rate and pattern of decomposition
There are several factors generally affecting human body decomposition, depending on the circumstances surrounding a death. The ambient temperature is probably the most important factor affecting decomposition since a higher temperature increases bacterial growth and enzymatic function [Zhou and
Byard 2011, Campobasso et al. 2001]. The degree of decomposition at different anatomical sites may progress depending on trauma. Decomposition is often initiated where there are open wounds or injuries, for example burns, cuts, or tears. Internal organs may also decompose at a faster speed due to injuries to the skin and underlying soft tissue that allow entry of bacteria [Zou and Byard 2011, Tsokos 2004, Pinheiro 2006]. If the cause of death is infection or septicaemia, the body may decompose at a faster rate [Zou and Byard 2011, Tsokos 2004, Pinheiro 2006]. On the other hand, bacterial growth can be reduced through dehydration of the dead body, for example in a dry environment (i.e., low humidity) with a constant air flow inducing the mummification process [Campobasso et al. 2001, Tsokos 2004]. Ante-mortem treatment with antibiotics or a large loss of blood volume before death [Tsokos 2004] or poisoning with carbolic acid or strychnine [Javan et al. 2019] can reduce bacterial growth, resulting in a slower rate of decomposition. Thicker subcutaneous adipose tissue contains more water, which maintains the body temperature [Ellis 2000]. Individuals with little subcutaneous adipose tissue may therefore decompose at a slower rate than individuals with overweight [Matuszewski et al. 2014].

Human decomposition in an indoor setting

Forensic taphonomy studies have been carried out on human remains in the indoor setting, although studies in this specific environment are still rather uncommon [Galloway et al. 1989, Goff 1991, Schroeder et al. 2002, Ritchie 2005, Anderson 2011, Cockle 2013].

Disparities in decomposition rates between indoor and outdoor environments has been suggested. A study of human decomposition in southern Arizona indicated that remains deposited in closed environments decomposed more slowly during the initial phases of decay, but progressed to skeletonization stages quite rapidly [Galloway et al. 1989]. Galloway et al. [1989] describes one case of decay in an indoor setting during late summer were over fifty percent of the body became skeletonized within only seven days. The decaying bodies often reached skeletonization stages after four months, which can be compared with outdoor decomposition, where skeletonization did not occur until eight months after time of death [Galloway et al. 1989]. In enclosed environments, the study also indicated that the human remains were less prone to mummification, but rather underwent what the authors described as moist decomposition [Galloway et al. 1989]. In the cases with mummification, this occurred about two weeks later than mummification in an outdoor setting [Galloway 1997]. The climate in Arizona is characterised by hot and arid conditions, with a large differences in moisture between indoor/closed and outdoor/open environments.

Megyesi et al. [2005] studied both indoor and outdoor human decomposition, covering most regions of the United States. All cases displayed evidence
of insects’ access. The study indicated that the indoor cases did not stand apart from the outdoor cases [Megyesi et al. 2005]. However, the major part of the analysed human remains samples consisted of outdoor cases. Three studies [Ritchie 2005, Anderson 2011, Guerra 2014] indicated a slower decomposition rate indoors, in contrast to Cockle’s study [2013], which suggested that indoor cases commonly decomposed at a faster rate than outdoor cases regardless of season or temperature.

The effect of insects’ contribution to human decomposition within enclosed structures has not been fully quantified. Insects may not have access to the decaying body in an indoor setting, or access could be restricted. The temperature inside a closed structure may also differ from outside, which probably leads to inconsistency in the rates of insect development [Haskell 2006]. Insects are considered to be responsible for eliminating the majority of soft tissue and insect access to decaying human remains is therefore an important variable for determining the rate of decomposition. Insect activity was influenced by seasonal weather, accessibility of remains, and “location of the body” [Galloway et al. 1989, Mann et al. 1990]. The case report of Schroeder et al. [2002] also indicated a geographic effect on the type of insect species found in an indoor/closed setting. Simmons et al. [2010] stated that regardless of if a body was indoors, buried, or submerged, the presence or absence of insects had the largest impact on decomposition rate.

Hayman and Oxenham [2016b] published a longitudinal study of two donated human bodies which were monitored closely while decomposing in sequence in almost identical indoor settings. The two bodies decomposed at different rates, and the degree of decomposition varied greatly between them. The authors suggested that this large difference could be the result of perimortem disease treatment of one of the bodies in close proximity to death. The same authors [Hayman and Oxenham 2017] also presented a study of 239 human cases found indoors in several states of Australia and applied a new scoring-based method, with different stages and descriptions than those of Megyesi et al. [2005], but also called Total Body Score (TBS) but. Hayman and Oxenham’s scoring method was based on the assessment of decomposition of the brain, heart, liver, and spleen, in addition to an external appearance score. During the time span of 0 to 14 days post-mortem, it was possible to accurately estimate the time of death. Beyond this time, the variability of the body organ decomposition was too great, rendering any estimate less accurate [Hayman and Oxenham 2017].

Gelderman et al. [2018] also developed a new decomposition scoring method based on forensic cases (79 bodies found indoors and 12 found outdoors) in the Netherlands. The design of this scoring method was similar to that of TBS, also including three partial body scores (facial, body, and limbs). This new decomposition scoring method resulted in inaccurate PMI estimation in cases with short PMIs and high decomposition scores, as well as in cases with long PMIs (> 10 days).
Maile et al. [2017] investigated the universal equation to estimate PMI developed by Vass [2010] based on 19 indoor cases found in Nebraska and Hawai’i. In this study, the authors stated that the PMI estimates were accurate in 79% of the indoor cases. The equation (in which the degree of decomposition is expressed as percent of body surface) resulted in inaccurate PMI estimation in cases with soft tissue mass loss of > 20% and a PMI of ≥ 4 days.

Temperature and Accumulated Degree-Days

Several studies have made use of heat energy units, known as Accumulated Degree-Days (ADD), to quantify the rate of decomposition. ADD represents the accumulation of thermal energy needed for biological and chemical reactions in a decomposing body, or, in other words: the product of chronological time and temperature combined [Simmons et al. 2010]. To calculate ADD, the maximum and minimum temperatures on a day are averaged to produce the mean daily temperature, which is multiplied by the number of days at that temperature. Arnold [1959, 1960] was the first to introduce the concept of ADD as a measure of thermal units. ADD was later used as a measure of cumulative thermal energy to follow insect development [Edwards 1987]. Vass et al. [1992] modified ADD, defining it as the product of the average daily temperatures above zero degrees Celsius and the number of days that the dead body had been decomposing at each respective temperature.

The indoor climate

The indoor environment does not exhibit the same extreme seasonal and daily temperature fluctuations as the outdoor environment. In Sweden, the indoor climate is very well regulated. According to the Swedish construction standards, the lowest permitted temperature at floor level is 16 °C and the highest room temperature allowed during a heatwave is 28 °C. The recommended indoor temperature is between 20 to 23 °C [FoHMFS 2014:17]. For the most part, indoor environments are controlled and in line with the above regulations, although aberrations occur.

This temperature interval results in a limited ADD range for the forensic cases found decomposed in an indoor setting, as compared with those in an outdoor setting. How this may affect the methods for PMI estimation has not yet been explored.

Decomposition during morgue storage

The refrigerating effect in a morgue can keep bodies looking fresh for an extended period [Galloway et al. 1989]. However, it is not known at what temperature decompositional processes actually cease [Megyesi et al. 2005]. Vass
et al. [1992] have stated that decomposition will occur down to 0 °C and Micozzi [1991] stated that no decomposition would take place at temperatures lower than 4 °C.

Heat is also produced by larval masses [Mann et al. 1990, Haskell et al. 1997,] and in a few indoor cases with large masses of larvae, the heat they produced could be noticeable at the autopsy. The study of Johnson et al. [2013] suggested an increase in carcass temperature in the absence of larval masses or solar radiation, due to bacterial metabolism. Currently, the possibility of continued decomposition during storage in a morgue facility cannot be completely ruled out.

Quantifying the decompositional process

Several researchers have described the post-mortem changes taking place during the process of decomposition [e.g., Rodriguez and Bass 1985, Galloway et al. 1989, Mann et al. 1990, Bass 1997, Clark et al. 1997, Galloway 1997, Komar 1998]. However, the division of the decompositional process into several stages can only establish a wide time interval due to differences in environmental conditions [Megyesi et al. 2005]. Vass [2010] calculated the degree of decomposition as a percentage instead of assigning a specific stage. This percentage may be difficult to determine exactly based only on external decompositional changes.

Hayman and Oxenham [2016a] described two main approaches to quantifying the decompositional process, which have developed in recent years: 1) Establishing a method which incorporates the main variables affecting the decomposition (e.g., temperature, insect access, etc.). 2) Establishing a mathematical description of the entire decompositional process.

Two research groups were the first to link soft tissue loss due to decomposition and ADD [Vass et al. 1992, Vass 2010, Megyesi et al. 2005]. Human bodies were monitored across four seasons, from early decompositional changes to complete skeletonization, at a decomposition study facility in Tennessee, USA. PMI was converted into ADD, and the correlation of ADD with decompositional changes was followed. The human bodies in this outdoor setting became skeletonized at 1,285 ADD ± 110 [Vass et al. 1992]. Megyesi and colleagues [2005] studied forensic cases with known PMI, which were given a TBS assessing the decomposition stage, and then calibrated against the total of 1,285 ADD, producing a linear regression. It is argued that ADD better represents the decompositional process [Michaud and Moreau 2011]. In contrast, another study suggested that ADD does not provide the entire taphonomic story, i.e., the decompositional process appears to be too complex for universal modelling based on a single or narrow set of variables [Forbes et al. 2019].
The work of Megyesi and colleagues [2005] was a starting point for scoring-based methods in which the decompositional process was quantified by means of a specific TBS value reflecting how much decomposition had taken place overall. This effort to describe the decompositional process in a standardised way has resulted in several studies further exploring scoring-based methods and decomposition in different environments. Additional scoring-based methods have been developed by other researchers, but these have not gained the same broad impact on the field of forensic taphonomy as the TBS system. However, Megyesi et al. [2005] were not first to develop and present a standardised decomposition scoring method. In an article from 1982, Zumwalt et al. presented an objective scoring method for establishing the degree of putrefaction based on eight physical changes (skin slippage, mummification, changes in the eyes, marbling, rigor mortis, bloating, purging of fluids from mouth/nostrils, and discoloration). The main focus of this article was evaluation of how ethanol concentrations in decomposed human bodies correlated with degree of decomposition [Zumwalt et al. 1982].

Total Body Score method

The scoring method was first developed by dividing decomposition into four wide categories: no signs of decomposition (fresh, according to Megyesi et al.), early decomposition, advanced decomposition, and skeletonization. These categories were then subdivided into stages, describing the general appearance and characteristics of the body. Each stage was assigned a numerical value and since the stages of decomposition impact differently on different parts of the body, three separate scoring strategies were used: one for the head and neck, one for the trunk, and one for the limbs. The scores assigned to each anatomical region were then added together to produce TBS. A body lacking signs of decomposition has a TBS of 0 points and a completely skeletonized body has a maximum TBS of 32 points. When the decomposition stage varies across an anatomical area, the score assigned is the average of the two extremes observed within that area. Moffatt et al. [2016] modified and improved the Megyesi’s TBS method, rectifying some statistical errors (e.g., corrected the regression analysis, so that TBS was the dependent variable, not ADD). He also changed the TBS values so that the lowest point total is 0 instead of 3 (for the non-decomposition stage). Thus, the maximum is 32 points. Descriptions of the scoring are presented in Table 1.
Table 1. Total Body Score (TBS) scale and point values [Megyesi et al. 2005, Moffatt et al. 2016]. A body lacking signs of decomposition has a TBS score of 0, early decomposition stage TBS scores 1 to 13, advanced decomposition stage TBS scores 14 to 21, and skeletonization stage TBS scores 22 to 32.

<table>
<thead>
<tr>
<th>Score</th>
<th>Head and Neck</th>
<th>Trunk</th>
<th>Limbs</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>Fresh, no discolouration.</td>
<td>Fresh, no discolouration.</td>
<td>Fresh, no discolouration.</td>
</tr>
<tr>
<td>1</td>
<td>Pink-white appearance with skin slippage and some hair loss.</td>
<td>Pink-white appearance with skin slippage and marbling present.</td>
<td>Pink-white appearance with skin slippage of hands and/or feet.</td>
</tr>
<tr>
<td>2</td>
<td>Gray to green discolouration, some flesh still relatively fresh.</td>
<td>Gray to green discolouration, some flesh still relatively fresh.</td>
<td>Gray to green discolouration, marbling, some flesh still relatively fresh.</td>
</tr>
<tr>
<td>3</td>
<td>Discolouration and/or brownish shades, particularly at edges, drying of nose, ears, and lips.</td>
<td>Bloating with green discolouration and purging of decompositional fluids.</td>
<td>Discolouration and/or brownish shades, particularly at edges, drying of fingers, toes, and other projecting extremities.</td>
</tr>
<tr>
<td>4</td>
<td>Purging of decompositional fluids out of eyes, ears, nose, mouth, some bloating of neck and face may be present.</td>
<td>Post-bloating, following release of abdominal gases, with discolouration changing from green to black.</td>
<td>Brown to black discolouration: skin having a leathery appearance.</td>
</tr>
<tr>
<td>5</td>
<td>Brown to black discolouration of flesh.</td>
<td>Decomposition of tissue producing sagging of flesh, caving in of the abdominal cavity.</td>
<td>Moist decomposition with bone exposure in less than half of the area being scored.</td>
</tr>
<tr>
<td>6</td>
<td>Caving in of the flesh and tissues of eyes and throat.</td>
<td>Moist decomposition with bone exposure in less than half of the area being scored.</td>
<td>Mummification with bone exposure in less than half of the area being scored.</td>
</tr>
<tr>
<td>7</td>
<td>Moist decomposition with bone exposure in less than half of the area being scored.</td>
<td>Mummification with bone exposure in less than half of the area being scored.</td>
<td>Bone exposure in over half of the area being scored, some decomposed tissue and body fluids remaining.</td>
</tr>
<tr>
<td>8</td>
<td>Mummification with bone exposure in less than half of the area being scored.</td>
<td>Bones with decomposed tissue, sometimes with body fluids and grease still present.</td>
<td>Bones largely dry, but retaining some grease.</td>
</tr>
<tr>
<td>9</td>
<td>Bone exposure in more than half of the area being scored, with greasy substances and decomposed tissue.</td>
<td>Bone exposure with desiccated or mummified tissue covering less than half of the area being scored.</td>
<td>Dry bone.</td>
</tr>
<tr>
<td>10</td>
<td>Bone exposure in more than half of the area being scored, with desiccated or mummified tissue.</td>
<td>Bones largely dry, but retaining some grease.</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Bones largely dry, but retaining some grease.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Dry bone.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Two studies [Dabbs et al. 2016, Nawrocka et al. 2016] indicated a high interobserver reliability of the TBS method. However, concerns about the validity of the TBS approach in estimating a PMI have been raised [e.g., Myburgh et al. 2013, Suckling et al. 2015, Wescott et al. 2018; see also Table 2]. Based on the TBS method, modifications have been presented for submerged bodies [Heaton et al. 2010], charred bodies [Gruentahl et al. 2012], and bodies after hanging [Lynch-Aird et al. 2015]. Only 11 of 68 cases in Megyesi et al. [2005] were found in an indoor setting. These few cases were removed when Moffatt et al. [2016] presented the improved TBS method.

As presented in Table 2, many articles and theses has been published after the original study of Megyesi et al. [2005]. Table 2 illustrates the first 10 years, 2005 to 2015. Conference abstracts and posters have not been included. The studies can be divided into two types of research: PMI estimation methods or analyses of different factors affecting decomposition (i.e., decay rate). The majority of studies concern decay rate. One study investigated both PMI estimation and the rate of decomposition. A comprehensive selection of factors affecting the rate of decomposition is assessed using the TBS or a modification based on TBS (i.e., charred bodies or bodies after hanging). Several different investigation methods are used, usually based on human (forensic cases and donated bodies), pig, rabbit, rat, or mouse. The countries involved are the US, England, South Africa, and Poland, covering many different environments. Human remains found in an outdoor setting and placed directly on the ground surface are the most common, followed by remains exposed to burial or submersion. The popularity of the TBS method does not seem to be declining. However, not all taphonomic research is carried out using this method. While TBS may not the optimal method, as indicated by several studies (Table 2), it is easier to compare different studies with one another with a basis in a standardised way of assessing the decompositional process. The concept of TBS may be possible to improve and further develop, to better reflect the decompositional process in various settings.

Table 2. Previously published TBS studies during the years 2005 to 2015. The studies are longitudinal observation studies, excepting those carried out by Megyesi et al. 2005, Heaton et al. 2010, and De Donno et al. 2014, which are of a retrospective design. In the study by Megyesi et al. 2005, the majority of the cases were from Indiana and Illinois.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Model</th>
<th>Area</th>
<th>PMI</th>
<th>Decay rate</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Megyesi et al. 2005</td>
<td>Human (n = 68)</td>
<td>US</td>
<td>x</td>
<td>x</td>
<td>Included 11 indoor cases.</td>
</tr>
<tr>
<td>Adlam and Simmons</td>
<td>Rabbit (n = 24)</td>
<td>East England</td>
<td></td>
<td></td>
<td>Effects of repeated physical disturbance.</td>
</tr>
<tr>
<td>Schiel 2008</td>
<td>Pig (n = 10)</td>
<td>Indiana, Iowa, US</td>
<td>x</td>
<td></td>
<td>Supports Megyesi’s method.</td>
</tr>
<tr>
<td>Authors</td>
<td>Species (n =)</td>
<td>Location</td>
<td>Method/Outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>---------------</td>
<td>-------------------------------</td>
<td>----------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parsons 2009</td>
<td>Pig (n = 2)</td>
<td>Montana, US</td>
<td>x</td>
<td>Cold temperatures and arid conditions. Supported the use of ADD in different climates.</td>
<td></td>
</tr>
<tr>
<td>Dautartas 2009</td>
<td>Human (n = 6)</td>
<td>Tennessee, US</td>
<td>x</td>
<td>Effect of various coverings. Observed decompositional changes did not conform well with TBS. Supported Megyesi’s method. Formulae based on seasonal data more accurate.</td>
<td></td>
</tr>
<tr>
<td>Myburgh 2010</td>
<td>Pig (n = 30)</td>
<td>South Africa</td>
<td>x</td>
<td>Effects of penetrative trauma.</td>
<td></td>
</tr>
<tr>
<td>Cross and Simmons 2010</td>
<td>Pig (n = 34)</td>
<td>Northwest England</td>
<td>x</td>
<td>Effects of insect access.</td>
<td></td>
</tr>
<tr>
<td>Bachmann and Simmons 2010</td>
<td>Rabbit (n = 60)</td>
<td>Northwest England</td>
<td>x</td>
<td>Effects of insect access.</td>
<td></td>
</tr>
<tr>
<td>Simmons et al. 2010</td>
<td>Rabbit (n = 60)</td>
<td>Northwest England</td>
<td>x</td>
<td>Modification of TBS, created a Total Aquatic Decomposition Score TADS.</td>
<td></td>
</tr>
<tr>
<td>Heaton et al. 2010</td>
<td>Human (n = 187)</td>
<td>Northwest England</td>
<td>x</td>
<td>Bacterial succession, partial remains. TADS was indicated to be inadequate.</td>
<td></td>
</tr>
<tr>
<td>Dickson et al. 2011</td>
<td>Pig (n = 3)</td>
<td>New Zealand</td>
<td>x</td>
<td>Supported quantitative approach.</td>
<td></td>
</tr>
<tr>
<td>Parks 2011</td>
<td>Human (n = 1)</td>
<td>Texas, US</td>
<td>x</td>
<td>Effects of carcass mass.</td>
<td></td>
</tr>
<tr>
<td>Gruentahl et al. 2012</td>
<td>Pig (n = 48)</td>
<td>Northwest England</td>
<td>x</td>
<td>Bacterial succession. Controlled laboratory setting. Validation of previous study (Myburgh 2010). Did not support Megyesi’s method. Supported the TADS/ADD method. Effects of carcass size.</td>
<td></td>
</tr>
<tr>
<td>Myburgh et al. 2013</td>
<td>Pig (n = 16)</td>
<td>South Africa</td>
<td></td>
<td>Did not support Megyesi’s method. Avian scavenging and mummification.</td>
<td></td>
</tr>
<tr>
<td>Humpherys et al. 2013</td>
<td>Piglet (n = 9)</td>
<td>California, US</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sutherland et al. 2013</td>
<td>Pig/piglet (n = 45)</td>
<td>US</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Teo et al. 2013</td>
<td>Rabbit (n = 12)</td>
<td>Malaysia</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White 2013</td>
<td>Pig (n = 3)</td>
<td>Montana, US</td>
<td>x</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Assessing taphonomic data and reporting PMI estimates

In forensic medicine, conclusions based on probability are common. The validity of these conclusions is dependent on their foundations, which is many times based solely on personal experience. Two studies have evaluated forensic physicians’ PMI estimates based on visual assessment of the decomposition of human remains. Both studies indicated a poor correlation between the estimated PMI and the true PMI [Aydin et al. 2010, Gelderman et al. 2019]. PMI estimates based only on an individual forensic physician’s experience of assessing the decompositional process seem to be unreliable as evidence. It would be possible to assess probability associated with evidence based on the findings of the forensic autopsy. An example could be quantification of the decompositional changes (i.e., taphonomic data). By providing an evidence-based foundation, it might be possible to increase the validity of PMI esti-

<table>
<thead>
<tr>
<th>Study</th>
<th>Specimen</th>
<th>Environment</th>
<th>Location</th>
<th>PMI Method</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Donno et al. 2014</td>
<td>Human (n = 68) Submersion</td>
<td>Adriatic Sea</td>
<td>x Did not support TADS/ADD method in cold temperatures.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matuszewski et al. 2014</td>
<td>Pig (n = 24) Surface</td>
<td>Western Poland</td>
<td>x Effects of body mass and clothing.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guerra 2014</td>
<td>Human (n = 80) Surface/indoor/submersion</td>
<td>Pennsylvania, US</td>
<td>x Region-specific standards for decomposition stages indicated as estimating ADD more accurately than TBS method. Included 37 indoor cases.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forman 2015</td>
<td>Human (n = 6) Surface</td>
<td>Tennessee, US</td>
<td>x Bodies wrapped in plastic. Did not support Megyesi’s method.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suckling et al. 2015</td>
<td>Human (n = 10) Surface</td>
<td>Texas, US</td>
<td>x Did not support Megyesi’s method.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roberts &amp; Dabbs 2015</td>
<td>Pig (n = 16) Surface</td>
<td>Illinois, US</td>
<td>x Frozen versus never frozen bodies.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MacDonald 2015</td>
<td>Mouse (n = 100) Burial</td>
<td>Southwest England</td>
<td>x Effects of cyanide poison. Controlled laboratory setting.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Card et al. 2015</td>
<td>Pig (n = 20) Surface</td>
<td>Northwest England</td>
<td>x Effects of clothing.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
mates, as well as assessing the strength of evidence. Objectiveness and transparency are of great importance when reporting PMI estimates. Police investigators or courts may be misled if a forensic expert is not able to both estimate and communicate the degree of uncertainty regarding a specific PMI estimate.

Usually, PMI estimation provides a point estimate or confidence interval for the time of death. However, this is not always the optimal way to present the results of the PMI estimation in a specific forensic case. For example, if a witness or defendant states that a person was alive at a given timepoint, the forensic evidence, i.e., taphonomic data such as the degree of decomposition, should be possible to evaluate in relation to this statement, in combination with other available evidence. In this specific case, an answer in the form of upper and lower bounds of a confidence interval may not necessarily be informative, or could even be misleading. In the case of a police investigation of suspected crime, it is of importance to have valid standards for interpreting and weighting forensic evidence [Koblentz 2010]. Misunderstandings related to statistical presentations in courts have been revealed to cause severe miscarriages of justice. Therefore, there is a need to create guidelines for statistical evaluation of evidence [Aitken 2010a].

In forensic statistics, the Bayesian approach has been implemented by forensic institutes in many countries to evaluate forensic evidence [Aitken and Taroni 2004, Taroni et al. 2006, Aitken 2010a, Nordgaard et al. 2012] and has been applied to several different forensic matters. Examples include evidence based on DNA analysis or transfer of glass, fibres, and paint [Aitken 2010b], forensic age estimation [Sironi et al. 2017], PMI estimation based on forensic entomology [Andersson and Lindström 2015], as well as prediction of cause of death based on forensic autopsy [Yeow et al. 2014] and interpreting forensic toxicology to assess the likelihood of fatality [Langford et al. 2015].

The basic concept in Bayesian statistic is that of Bayes theorem, a mathematical formula used for calculation of conditional probability. In a Bayesian framework, our belief about the expected probability of obtaining a specific result is used to calculate the posterior probability of a hypothesis. The value of evidence expresses the extent to which the evidence would change our prior belief. The likelihood ratio corresponds to the value of evidence and could also be expressed as a qualitative statement on a verbal scale [Aitken 2010b, Nordgaard et al. 2012]. Probabilities are thought of as measurements of belief and the Bayesian approach allows combination of objective probabilities based on data as well as subjective probabilities based on knowledge and experience [Taroni et al. 2006]. Forensic caseworks vary significantly with respect to available information and the questions being addressed. A Bayesian approach to assessing and reporting PMI estimates could be of value in many different scenarios.
Aim of thesis

The overall aim of this thesis was to determine if taphonomic data derived from an indoor setting could expand our knowledge regarding the decompositional process per se, and act as a basis for scoring methods to improve the precision and accuracy of PMI estimates.

Aim of each study

In **Paper I**, the aim was to investigate whether the TBS method could achieve accuracy and precision in PMI estimation of decomposed human remains found in an indoor setting.

In **Paper II**, the aim was to determine if a novel scoring-based method for histological quantification of decomposed human livers could be a potential aid in increasing the precision of PMI estimates.

In **Paper III**, the aim was to determine if there was a relationship between microbial neoformation of volatiles and PMI, and if the volatiles could be used as a tool to improve the precision of PMI estimates of decomposed human remains in an indoor setting.

In **Paper IV**, the aim was to elucidate how a general likelihood ratio-based approach of comparing hypotheses can be used in the context of PMI estimation. Further, we aimed to overcome some of the current limitations associated with evaluation and interpretation of evidence in relation to PMI of decomposed human remains and its reporting to investigators and courts.
Materials and Methods

Selection of cases

A total of 590 forensic autopsy cases were scored prospectively at the routine forensic autopsy in accordance with the Megyesi et al. [2005] TBS system. The cases were compiled in a non-consecutive manner during 2010–2018 at the Department of Forensic Medicine in Uppsala, Sweden. A small sample of cases scored during 2016–2017 came from the Department of Forensic Medicine in Gothenburg, Sweden.

The overall inclusion criteria for this thesis were human remains discovered in an indoor setting (e.g., in an apartment or a house) with known PMI, from adults (> 18 years), without extensive trauma, animal scavenging, burns, or having been submerged (in a bathtub). A small body size (children), extensive trauma, or other major alterations to the dead body may affect the decomposition rate and pattern [Matuszewski et al. 2014, Pinheiro 2006, Mann et al. 1990, Heaton et al. 2010, Gruentahl et al. 2012].

In Paper I, the dataset consisted of 140 cases that meet the inclusion criteria. Of these 140 original cases, 82 cases were used in Paper II as a basis for the construction of the Hepatic decomposition score (HDS) system. In addition to the aforementioned inclusion criteria, the selected cases must also have assessable liver tissue samples. Many bodies in an advanced state of decomposition lacked available liver tissue sample. Some forensic cases had extensive steatosis and/or cirrhosis and were therefore not possible to assess. An additional 154 new cases with assessable liver samples were used in Paper II. The total of 236 included cases were divided into a training dataset (2/3 of the cases, including the 82 original cases) and a validation dataset (1/3 of the cases, only new cases). The sampling of the training and validation dataset was made to even out the seasonal distribution in the groups.

In Paper III, the selection of cases was made based on availability of analysed femoral vein blood (i.e., chromatograms from ethanol analysis). A total of 412 cases were included in this study. Of the original 140 cases in Paper I, 47 met the inclusion criteria, as well as 77 of the 154 new cases in Paper II. The remaining 288 cases were collected specifically for this study.

In Paper IV, a training dataset consisting of 93 cases without presence of insect activity was selected from the original 140 cases used in Paper I. Eight new indoor cases were also used to illustrate the Bayesian methodology presented in this study.
General methodology and study design

For each included forensic autopsy case, a protocol with body charts (Figure 1) was used when assessing and scoring the external decomposition. The body charts consisted of a front and back view of the body, which was divided into 32 regions (each region scored as separate unit) in this model. During forensic autopsies, each case was scored and the distribution of observed decompositional changes, as well as the presence of insect activity (when applicable) was noted on the body charts. Since it was initially not known if the TBS descriptions was optimal for the indoor cases, an additional system describing the post-mortem changes was developed and used. These post-mortem changes were as follow: livor mortis, presence of vibices, rigor mortis, greenish discoloration of skin, marbling of skin, skin blisters, skin slippage, decompositional fluids from mouth/nose, desiccation/mummification, bloating, liquefaction of the brain, decompositional fluids in body cavities, absence of heart blood and/or femoral vein blood, loss of soft tissue, bone exposure, and presence of insect activity. Figure 1 illustrates scoring and assessment of a fictive case with uneven decomposition. Further, information about indoor temperature, clothing, age, gender, body mass index, date the person was last seen alive, date of discovery, and date of autopsy was collected. Transport time from the discovery site to the morgue facility was also noted. The PMI of each case was calculated from the date the person was last seen alive (or based on other evidence such as daily newspapers or other contents of a mailbox, telephone calls, etc.). The ADD of each case was calculated in the following way: the PMI (in days) multiplied by temperature at the site when the body was discovered. In addition, the morgue time (the time from the date of discovery to the date of the autopsy) was multiplied by the temperature in the morgue’s refrigerator. An example follows: (10 days x +22 °C) + (5 days x +5 °C) = 245 ADD (total).

After each autopsy, the completed protocol with body charts was reviewed and checked against the final autopsy report and the police report. The partial body scores (head, trunk and limbs), and subsequently TBS, were calculated based on the 32 scored anatomical regions. The information was compiled in an Excel file.

The original TBS method was slightly modified. In this model, 32 regions were scored separately, making it somewhat more detailed than the original method of Megyesi et al. [2005]. Also, each case was scored prospectively at the autopsy, while cases were scored retrospectively from photographs in Megyesi’s study. The partial body scores were calculated in a similar way as in Megyesi et al. However, a mean was calculated, since the indoor decompositional changes may be unevenly distributed. For example, there can be apparent differences between the front and back view of the trunk. Further, in this thesis, the modification made by Moffatt et al. [2016], starting the TBS scale at 0 and ending it at 32, was used. The original descriptions given by Megyesi et al. [2005] were used (Table 1 in the Introduction section).
The assessments of liver tissue samples and the chromatograms from ethanol analysis of femoral vein blood were performed retrospectively. The processing and staining of liver slides, as well as the analysis of ethanol levels, were performed by professionals at our department and at the Department of Forensic Toxicology in Linköping, Sweden, respectively, as part of the routine forensic investigation. The liver slides were later collected from our department’s archive and examined under a light microscope. The liver and femoral vein blood samples were collected as part of the routine autopsy procedure. No liver sample or femoral vein blood was taken specifically for the studies in this thesis.

![Figure 1](image.png)

**Figure 1.** A schematic presentation of a fictive indoor case with unevenly distributed external decompositional changes illustrating the protocol with body charts that was used when scoring and assessing forensic autopsy cases.

All four papers were of a methodological nature. **Paper I** investigated the usability of the well-established TBS/ADD method in an indoor setting. **Paper II** focused on development of a novel scoring-based method for quantification of decompositional changes and as a possible aid in PMI estimation. The method used defined histological scores based on the progression of decomposition in the human liver. Initially, several organs and tissues were of interest, such as the heart, skin, skeletal muscle, etc. A pilot study indicated that the liver was the most promising of the tested tissues/organs with a well-defined histology, which was why it was selected. Each of the 82 liver samples was examined several times before the HDS system was finalised. Then, the
cases were revaluated and given a final score. The HDS system was further used in a blinded assessment of an additional 154 liver samples. **Paper III** investigated the presence of neoformated ethanol, N-propanol, 1-butanol, and acetaldehyde, and their relationships to the degree of decomposition (TBS) and PMI. The chromatogram of each case was retrospectively assessed and the relative amounts of detected volatiles were calculated using the ratio between the peak heights for each detected substance and the internal standard (tert-butanol) as a proxy. **Paper IV** focused on the construction of a Bayesian framework and its application for interpretational purposes and for reporting PMI estimates.

### Statistical analyses

R (https://www.r-project.org/) together with R libraries were the main software and computational tool used for statistical analysis. Microsoft Excel 2016 was generally used for database handling and organisation, as well as for descriptive statistics.

In **Paper I**, some of the improvements suggested by Moffatt *et al.* [2016] in their work based on Megyesi’s original data were applied. A linear regression method was used, where TBS was plotted against log_{10}ADD. The Box-Cox transformation of TBS was investigated for possible use. The difference between estimated and true log_{10}ADD was presented as a Bland-Altman plot. In the study, the *inverse prediction* method was used in order to calculate a PMI, comparing it with the true PMI of the case. Moffatt’s inverse prediction method is considered somewhat statistically doubtful in use. However, we wanted to make a comparison with this published method and therefore performed the calculations. The dataset of 140 forensic autopsy cases was divided into groups based on presence or absence of insect activity or desiccation, and also into spring-summer or fall-winter cases. The distribution of TBS and the post-mortem body mass index (BMI) was investigated. Seasonal effects were also investigated in the complete dataset by using the following function: TBS log_{10}(ADD) + sin(2PI*month/12) + cos(2PI*month/12).

In **Paper II**, the Box-Cox transformation of HDS was used to improve the fit of the model. We established a stochastic model by relating the variables of interest. In this paper, these variables were the HDS markers and/or the partial body scores (head, trunk, limbs) and the log_{10}ADD. A multivariate normal regression model was applied in order to compute the *likelihood function*. The maximum likelihood (ML) algorithm was used and the fitted model was tested in a validation dataset. The inter-observer reliability of HDS was also tested during the development of HDS and later in the finalised version. Data analysis used intra-class correlation (ICC) and standard error of measurement (SEM).
In **Paper III**, the occurrence of ethanol, N-propanol, 1-butanol and acetaldehyde was studied. To investigate the association between PMI or TBS and the detected volatiles (ethanol, N-propanol, 1-butanol, and acetaldehyde), the Pearson correlation test and linear regression of the \( \log_{10}(\text{relative amounts of the volatiles}) \) were performed. Further, the TBS/ADD method was investigated, using the four volatiles as factor variables in the linear regression, i.e., presence or absence in the femoral vein blood. The residual standard error was used as an indicator of the model’s precision.

In **Paper IV**, the constructed Bayesian framework was applied in order to extend the well-known likelihood ratio method to situations where PMI hypotheses provide a range for the time of death rather than a timepoint. Different prior probability distributions (priors) were specified. A stochastic model was used where the likelihood curves were obtained from partial body scores. When training the multivariate regression model, the expectation maximisation (EM) algorithm was used for the main model and as an alternative method to the ML algorithm. The EM algorithm made it possible to account for the uncertainty of the PMIs in the training cases when the model was fitted, while the ML algorithm used a point estimate for PMI. To test if the models produced reasonable estimates of PMI and uncertainty of these estimates, the training and prediction procedure was performed through leave-one-out cross-validation.

**Ethical considerations**

The datasets used in Papers I to IV consisted only of non-sensitive information and it is not possible to identify individual persons from the presented data. The General Data Protection Regulation applies only to living individuals and is therefore not applicable to these studies.

The research carried out was aimed at method evaluation and development of novel methods with emphasis on improving the forensic (medico-legal) investigation. Assessment of post-mortem changes is part of the routine examination carried out by a forensic pathologist. Chromatograms and liver tissue slides were originally processed for the purpose of forensic investigations, in accordance with Swedish law. The value of having a more reliable and accurate method for estimation of PMI is high, not only for the police, but also for society in a legal context, as well as for the close relatives of a deceased person.
Results

Paper I

Indoor decomposition

The majority of cases (68%) found in an indoor setting were without presence of insect activity. Desiccation was present in 16% of the complete dataset and more frequent in cases with PMI of more than 35 days. Many of the cases (49%) were discovered during the summer months (in Sweden: June, July and August) and, as expected, insect activity was evident during this time period (as seen in Paper I, Fig. 1).

Figure 2. Example of indoor decomposition. The upper row (cases A to C) illustrates moist decomposition and the middle (case B) and right photos (case C) also the presence of insect activity. The lower row (cases D to E) illustrates desiccation (without presence of insect activity). The PMIs of the cases were: 2 days (A), 8 days (B), 11 days (C), 19 days (D), 30 days (E), and 50 days (F). Photos taken at autopsy by Ann-Sofie Ceciliason.

The decompositional process in an indoor setting was observed to consist of two major categories: (i) Moist decomposition with skin slippage (including hair loss and degloving), bloating, and liquefaction of the soft tissues, and (ii) desiccation with dry, intact skin and the drying out of soft tissues and internal organs. In addition, bodies showing a combination of both categories were
observed. There were differences apparent between the ventral and the dorsal parts of some decomposed bodies, due to position after death. For example, the part of the body in contact with a surface, e.g., a floor, could display moist decompositional changes, while the exposed part of the body was desiccated. The presence of insect activity was found in both categories, although insect larvae were more frequently found in cases with moist decomposition.

As seen in Figure 2, the PMI differs considerably between cases of moist decomposition and cases of desiccation. Case A displayed marbling and greenish discolouration of the skin, as well as bloating. Purging of decompositional fluids from mouth and nostrils was prominent (not visible in photo, PMI 2 days). In case B, the head and trunk were covered in insect larvae. When the insect larvae were removed a pale greyish pink to brownish discolouration was visible, as well as skin slippage. The case did not display bloating (PMI 8 days). Case C had a similar amount of insect larvae as case B. Extensive soft tissue loss within the head, neck, and upper torso and exposed bones were seen. Heart and lungs were missing (PMI 11 days). In case D, bloating was present and the dorsal part of the body displayed moist decomposition with skin slippage and purging of fluids. Desiccation was evident within the ventral parts of the body, i.e., face, arms, thighs, and abdomen. The intact skin displayed a greenish yellow to orange colour with dark green and distinct marbling within the desiccated areas (PMI 19 days). In case E, the desiccated areas were darker orange. Some green discolouration was seen in areas with moist skin. White areas with mould were scattered across the body (PMI 30 days). Case F displayed a dark reddish brown, desiccated, and leather-like skin. The underlying soft tissue was also desiccated, making the body stiff. Still, there were some minor areas with pale and moist skin, especially in areas that had been in contact with a hard surface (PMI 50 days).

Two main types of desiccation were observed in the indoor cases: brown or black discolouration and leather-like skin resembling case F or yellow to orange translucent and parchment-like skin resembling cases D and E. A wide range of patterns and different combinations of the two main types were seen. The post-mortem body mass index (BMI) of the desiccated cases differed greatly within the group. Many of the cases with apparent overweight (i.e., high BMI) displayed the desiccation type with yellow to orange translucent and parchment-like skin. The underweight cases were more prone to brownish or black discolouration and leather-like skin, and a general stiffness of the body indicative of drying out of the soft tissue.

Statistical analysis

The distribution of TBS and post-mortem values of BMI was illustrated using boxplots (in Paper I, Fig. 2). The 140 cases were divided into four subgroups based on the presence or absence of desiccation (des±) and insect activity (ins±). The most apparent difference between the subgroups was that
(ins+/des+) displayed higher TBS and greater agreement between cases, with a narrow range of TBS, compared with the other subgroups. However, this subgroup was very small (n = 5). The opposite was seen in the (ins+/des-) group with a very large range of TBS and lesser agreement between the observed cases. The BMI within the (ins-/des-) group displayed the widest range of the four groups. In the (ins+/des-) group, one outlier was found with extremely low BMI of 4.9 kg/m² (in Paper I, Fig. 2) possibly signifying extensive soft tissue mass loss.

Figure 3. TBS vs. PMI in the presence or absence of desiccation (des ±) and insects (ins ±). Four logarithmic trendlines, one corresponding to each group, are shown.

In Figure 3, the scatterplot illustrates the relationship between TBS and PMI in the presence or absence of desiccation (des ±) and insect activity (ins ±). In general, cases with insect activity (ins+/des-) had a higher TBS and shorter PMI (as also illustrated by case A to C in Figure 2). Cases without insect activity (ins-/des-), were rather gathered in the TBS range 5–15 and PMI 0–30 days. Cases with desiccation (ins-/des+) were mostly in a TBS range slightly lower than cases with insect activity, but often with a longer PMI. The small group of 5 cases with both insect activity and desiccation (ins+/des-) were too few to draw any certain conclusions.

The relationship between TBS and ADD was evaluated in a linear regression model plotting log_{10}ADD against TBS. In the complete dataset (n = 140), the coefficient (r² value) was 0.55. In the cases without insect activity (ins-),
the $r^2$ value increased to 0.66, but it decreased to 0.45 in the cases with insect activity ($ins+$). The possible effect of seasonal distribution was then investigated. We divided the cases with or without presence of insect activity into spring-summer and fall-winter cases. As illustrated in Paper I, Fig. 3, the linear regression model resulted in low $r^2$ values of 0.36 ($ins+$) and 0.59 ($ins-$), respectively, during spring-summer time. Higher $r^2$ values were noted during fall-winter time of 0.73 ($ins+$) and 0.76 ($ins-$), respectively. However, the cases with presence of insect activity ($ins+$) were scarce during fall-winter. To further analyse a possible seasonal effect on the relationship between PMI and ADD, the seasonal variation was modelled as a sine function (representing the seasonal changes in temperature during a year). In the complete dataset, the cosine term was significant ($p = 0.05$). However, when the cases with or without presence of insect activity were analysed separately, no significant effect was noticeable.

Both Megyesi et al. [2005] and Moffatt et al. [2016] used Box-Cox transformation of TBS. In our study transformation of TBS was also carried out. The best fit of the model in the indoor dataset, was obtained with exponents around 1. It was therefore decided to further on continue with the untransformed TBS (i.e., exponent 1) in the study.

The dataset (grouped into cases with or without insect activity) was compared with that of Megyesi et al. [2005] original dataset (Paper I, Fig. 5). An apparent difference between the datasets were noted. Megyesi’s data expressed somewhat higher TBS overall and the outdoor cases were relatively well separated from the indoor cases. Indoor cases with prominent effects of insect activity were mainly found close to Megyesi’s datapoints. The small group of 11 indoor cases in Megyesi’s data were all closer to our indoor cases with insect activity ($ins+$) than to the cases without insect activity ($ins-$) in the linear regression model (see Paper I, Fig. 5).

Lastly, the precision of the model was tested. Estimation of PMI was performed using the inverse prediction method suggested by Moffatt et al. [2016]. There was assumed to be a roughly linear relationship between $\log_{10}$ADD and TBS in our dataset. This would also mean that we had a constant width of the $\log_{10}$ADD interval where we could expect to observe a specific TBS value. The Bland-Altman plot was created to test this assumption. As indicated by this plot (Paper I, Fig. 6, difference between estimated $\log_{10}$ADD and true value), the uncertainties in the estimation were constant on the log scale and there were no apparent systematic errors (i.e., the datapoints were symmetrically distributed around zero, for all magnitudes of ADD). The assumption was therefore found to be valid. In order to make inferences about the distribution of $\log_{10}$ADD (or PMI) for a new case, the function from the fitted models could be reversed (i.e., inverse prediction), as described by Moffatt et al. [2016]. Following this procedure, we had to assume that the new case was a sample from a population where any PMI value was equally likely. The scatterplots in Paper I, Fig. 7 show the correlation between the estimated
PMI and the true PMI. As seen, there were several outliers and a rather large spread of values, i.e., a low correlation. The precision of the model was thus low in our dataset.

**Paper II**

The overall changes observed throughout the trajectory of the decompositional process in the liver were as follows: the hexagonal pattern, normally representing the hepatic lobules, declined progressively. The alignment of hepatocytes in cords was lost. Cell debris derived from disintegrating hepatocytes and stroma cells became evident. Later, loss of cell material was observed during liquefaction of the tissue. The cell nuclei of the hepatocyte went from distinct to darken and dense, and later ended up as a nuclear shadow. The collagen in the portal triad slowly disintegrated. Bile ducts and blood vessels within the portal triad diminished. The end stage was reached when only collagen remained and the tissue no longer was possible to identify as of liver origin. In Figure 4, the trajectory of liver decomposition is shows (see also Paper II, Table 1, Fig. 3 and 4).

In most cases with PMI less than 1 day, no changes were observed. A few cases with PMI of 1 to 4 days could also be without observable changes. Still, one case with a short PMI of 3 days had an advanced decomposition similar to what was seen in many cases with a longer PMI of > 20 days.

The detachment of hepatocytes from each other started during the first 1–5 days after death and were extensive at 10–20 days after death. Later, loss of cell material was observed due to liquefaction of the tissue (as seen in Figure 4 E). In most cases this loss of cell material was noticeable after PMI of 20 days. After PMI of 20 or more days, it was difficult to distinguish blood vessels or bile ducts in the portal triads. Later, after PMI of 30 days, blood vessels and bile ducts were no longer possible to identify with certainty. The end stage, when only scattered collagen was seen, was reached at around PMI of 35 days. Still, some cases (n = 12) with PMI of 43 to 217 days had a slightly less decomposed liver. Common for these cases was extensive external desiccation.

**The Hepatic Decomposition Score**

The newly created hepatic decomposition scoring system encompasses five HDS markers: cell nuclei and cell structure of the hepatocyte, bile ducts, portal triad, and architecture. The scale for each marker starts at 0 and goes to 4 (cell nuclei, bile ducts) or 5 (cell structure, portal triad, and architecture). Differences in the degree of decomposition within a liver sample are considered as separate scores and an average value is calculated. During the development of HDS, it was noted that the different structures in the liver decomposed at
individual paces. Therefore, the HDS markers were scored separately. The stages of and descriptions for the HDS system are presented in *Paper II, Table 2.*

**Figure 4.** The observed changes throughout the trajectory of decomposition in the human liver. Picro-Mallory stain, 200x. 

**A:** Distinct hepatocytes and alignment of hepatocytes in cords. 

**B:** Hepatocytes start to detach from each other. The cell nuclei of hepatocytes become darker and denser. 

**C:** The hepatocytes are detached from each other and scattered among cell debris. The hepatocytes have shadow nuclei. 

**D:** More cell debris is observed and the scattering of hepatocytes is more prominent. The hexagonal shape of lobules is lost. 

**E:** Distorted hepatic pattern with scattered remnants of hepatocytes. Cell debris is present in various amounts. The cell nuclei of the hepatocytes are not identifiable. 

**F:** No hepatic pattern is visible. Only scattered and fragmented collagen remains.
Statistical analysis

To investigate the reliability of HDS system, three independent observers were given randomly selected samples of 40 cases for blinded assessment. The observers had no prior knowledge of the HDS system. As support, they had the descriptions (as seen in Paper II, Table 2) and photographs of the original cases. They scored the liver slides with good (> 0.60) to excellent (> 0.75) ICC inter-observer agreement (Paper II, Table 3).

Different exponents for Box-Cox transformation were evaluated for the five HDS markers in the training data, to achieve an optimal fit of the model. For each HDS score, the model with best p value (i.e., when the model best explained the observed data) was selected (in Paper II, Fig. 5). Log_{10}ADD was plotted against the untransformed and the Box-Cox transformed HDS markers (Paper II, Fig. 6). After this, the maximum likelihood method was used in training the models. Three different models were applied consisting of (i) five HDS markers, (ii) three partial body scores, and (iii) HDS markers and partial body scores combined. The fitted models were used to calculate the value of log_{10}ADD of the highest likelihood in the training dataset (i.e., at which ADD it was most likely to observe this set of HDS markers or partial body scores). The true value of log_{10}ADD was plotted against the estimated value (Paper II, Fig. 7A). The fitted models were then applied to the validation data (Paper II, Fig. 7B).

Further, evaluation was made of how many of the predicted values of log_{10}ADD (i.e., the value with the highest likelihood) were within the 95% confidence interval specified by the actual or true log_{10}ADD of the specific case and the standard deviation (SD) derived from the model. The model based on HDS markers resulted in a maximum SD of 0.454. The model based on the partial body scores resulted in a SD of 0.358. A slight improvement was seen in the model combining HDS markers and partial body scores, resulting in a SD of 0.328. A comparison was also made of how many cases were found within the confidence interval between the three models. The three models performed rather similarly in the training dataset and the validation dataset. The model with only HDS markers in the training and the validation dataset reached 95% (representing two SDs) and the model with only partial body scores reach 96%. The combined model reached 96% for training data and 97% for validation data.

The HDS markers appeared to have slightly higher variance than the partial body scores. Notably, it appeared that the covariance between the HDS markers and the partial body scores was low. Within the set of HDS markers or partial body scores, the covariance was relatively high (Paper II, Table 5).

Some outliers could have a rather large impact on the estimated SD and the precision of the model. Excluding those cases resulted in a model with significantly narrower intervals and smaller SD, i.e., better precision. In approximately 34% of the 236 cases, external desiccation was present. The possible
effect of desiccation on the model’s precision was also tested. In the group with external desiccation, a greater number of cases with extended PMIs were found (i.e., generally higher ADD), when compared with the group without desiccation. Box-Cox transformation of the HDS markers resulted in clear differences in the optimal exponent between cases with or without desiccation (results not shown). Therefore, the significance of this test of the model performance was difficult to evaluate.

Paper III

Chromatograms from ethanol analysis (femoral vein blood samples) of the 412 forensic autopsy cases were evaluated retrospectively. An example of a chromatogram is seen in Figure 5. Volatiles of interest were ethanol, N-propanol, 1-butanol, and acetaldehyde. Microbial neoformation was classified based on the presence of N-propanol and/or 1-butanol in the femoral vein blood sample. If ethanol was present in the same sample as N-propanol and/or 1-butanol, the detected ethanol was assumed to be partly or entirely post-mortem neoformated. In the complete dataset, acetaldehyde was the most common finding, seen in 99% of the cases with microbial neoformation (n = 91) and 79% of the cases without microbial neoformation (n = 321). Ethanol was detected in 37% of the cases, N-propanol in 21% of the cases, and 1-butanol in 4% of the cases.

The majority of cases with external decomposition were within the lower range of PMI. Only 5 cases had a PMI > 30 days. Furthermore, the majority of cases were also within the early decomposition stage (greenish discoloration of skin, marbling, and skin slippage). However, some cases with decompositional changes within bloating stage were also present. Within the dataset, there were only two cases with higher TBS scores: one case within the active decomposition stage and the other in transition into the advanced decomposition stage with presence of partial desiccation.

The 412 cases were divided into two groups: with or without external decompositional changes. Microbial neoformation was indicated in 42% of the decomposed cases (n = 203), compared with 3% of the non-decomposed cases (n = 209). In total, 22% of all cases had signs of microbial neoformation. The degree of decomposition, measured as TBS, differed significantly between the non-neoformation group and the neoformation group. The observed TBS was evidently higher in the neoformation group, even if some outliers with higher TBS were also seen in the non-neoformation group (Paper III, Fig. 1A). Acetaldehyde was detected in an elevated relative amount in the decomposed group, compared with the non-decomposed group (Paper III, Fig. 1B). Moreover, the relative amount of acetaldehyde within the neoformation group was generally higher in the cases displaying external decompositional changes. However, a few cases (n = 6) in the neoformation group were without external
decomposition, while the majority (n = 85) displayed decompositional changes to varying degrees (Paper III, Fig. 1C).

Figure 5. A schematic representation of a chromatogram with N-propanol, 1-butanol, ethanol, and acetaldehyde (IS: tert-butanol). The relative retention times for each substance are used for identification.

Relationship between detected volatiles and TBS or PMI

Cases with microbial neoformation (i.e., presence of N-propanol and/or 1-butanol) were adjusted for suspected ante-mortem intake of alcoholic beverage. Information concerning signs of drinking (e.g., witness statements or many bottles/canisters of alcoholic beverage near the dead body) in the police report was used, as well as information in the death certificate (i.e., cause of death or contributing to the death was ruled as alcohol intoxication). The remaining cases (n = 56) were assumed to have only volatiles of post-mortem origin. This specific group was statistically analysed for possible relationship between the log10 relative amounts of the four volatiles and the degree of decomposition/TBS or the log10PMI Pearson correlation, and linear regression was applied. The result indicated a weak to moderate relationship between the detected volatiles and TBS (Pearson r 0.40–0.53), and weak relationship to PMI (Pearson r 0.06–0.33). Linear regression resulted in low R2 values (0.16–0.29), but high significance levels (with the exception of 1-butanol) for volatiles versus TBS and very low R2 values (0.0003–0.11) versus log10PMI (Paper III, Fig. 2).
The TBS/ADD method

The results did not support a direct linear relationship between the detected volatiles and TBS or PMI. Our proposed hypothesis based on these results is that the volatiles could be an indicator of decomposition rate. To determine if this hypothesis may be supported, further analyses were carried out using TBS as a function of ADD. In a linear regression model, the volatiles were included as factor variables, i.e., as presence or absence in a femoral vein blood sample. Only neoformated ethanol was included. Cases with blood samples positive for ethanol, but without signs of neoformation (i.e., negative for N-propanol and/or 1-butanol) were therefore excluded. The results indicated that a higher rate of decomposition was present in cases with microbial neoformation, i.e., at the same ADD, the observed TBS was higher than in a non-neoformation case. As illustrated in Figure 3, Paper III, 1-butanol seemed to be associated with a higher TBS and a higher decomposition rate, in comparison with the other volatiles.

The linear regression model of $TBS \sim \log_{10}ADD$ was compared with the neoformation linear regression model of $TBS \sim \log_{10}ADD + N$-propanol + 1-butanol + neoformated ethanol + acetaldehyde. The precision of the model increased when applying the neoformation model (i.e., the residual standard error was lower, at 1.75 versus 2.07). The regression models were repeated with $\log_{10}PMI$ instead of $\log_{10}ADD$ with a similar result, but a slightly higher residual standard error (results not shown). In the regression model, 1-butanol, neoformated ethanol, N-propanol, and $\log_{10}ADD$ or $\log_{10}PMI$ were significant.

Rate-modified $\log_{10}ADD$ model

Microbial neoformation might be used as a tool to improve the TBS/ADD method’s precision. The first step towards this was to use the relative amounts of neoformated ethanol, N-propanol, 1-butanol, and acetaldehyde, add them together, and calculate a $\log_{10}$volatiles. One could consider $\log_{10}ADD + \log_{10}$volatiles to be a rate-modified $\log_{10}ADD$, in which ADD not only represents the time x temperature, but also the rate of decomposition. When creating a linear regression based on TBS as a function of this rate-modified $\log_{10}ADD$, we could see an improved fit of the model, in comparison with the original $TBS \sim \log_{10}ADD$ model (Paper III, Fig. 4 A +B). In practical case-work, the coefficient in the linear regression model $TBS \sim \log_{10}ADD + \log_{10}$volatiles could be used for estimation of ADD, if both TBS and the relative amounts of volatiles are known.
Paper IV

Relationship between ADD and partial body scores

The relationship between accumulated temperatures (AT) expressed as ADD and partial body score is shown in Paper IV, Fig. 4. The curves represent the expected partial body score for each value of AT (ADD). The analysis indicated that the progression of decomposition was fastest in the head region (PBSH), followed by the limbs (PBSL) and trunk (PBST). The highest SD (1.28) was seen for PBSH, while PBST and PBSL both had a SD of 0.92. For interpretation proposes, we also studied how these parameter estimates influenced the likelihood curve. The SD depends only on which partial body scores are present or absent, not on the values of the partial body scores themselves. This means that the width of the likelihood curve, on a logarithmic scale, will be equal for all cases. The expected value of the normal distribution in the multivariate regression model corresponding to the likelihood function will thus be dependent on the observed partial body scores. In Paper IV, Table 1, the SD for the likelihood curve is shown, as well as the computed expected value for various subsets of partial body scores of a fictive case with PBSH 4, PBST 2.5, and PBSL 3.5. Eight new, unseen indoor cases were analysed to review the model’s performance. The results are presented in Paper IV, Appendix A3, Fig. S2. Generally, the limbs (PBSL) had the largest impact on the fitted models, while leaving out the head (PBSH) did not affect the model’s precision to the same extent.

Choosing a prior

To choose a prior distribution for the time of death, we first had to decide on the minimum and maximum values that we considered possible and thereafter the relative probabilities of time within this interval. In a case where the decomposed body has been identified and some information is available, for example when the person was last seen alive, this information can be taken as a maximum PMI. In this paper, four different priors were specified and used to illustrate the framework applicability.

If a decomposed body is unidentified and circumstances are unknown, i.e., there is no prior information, a natural choice of prior in one where time intervals of equal length would all be equally likely. This is a flat linear prior (uniform prior), represented by prior A in Paper IV, Fig. 2. In this type of model, it seems that an unrealistically high relative prior probability is given to very early values for time of death and, analogously, to very long PMIs. Instead, we choose to assume that any magnitude of PMI was equally likely and we could use a flat prior on log scale. This prior placed greater weight on later values for time of death, i.e., shorter PMIs. Further, we could also make assumptions about survival, i.e., a limited time span when a person had been
alive. This would make the time of death more likely to be towards the begin-
ning of the interval, rather than towards the end. This is represented by an
exponential survival prior called prior B in Paper IV, Fig 2. The last prior
used in this study was an exponential discovery prior, called prior C in Paper
IV, Fig. 3. This prior was based on the assumptions that the dead body was
found in a place where it was likely to be discovered. This would make the
time of death more likely to be towards the end of the interval, rather than
towards the beginning.

Likelihood ratios for competing hypotheses

Applying a Bayesian framework have us the opportunity to present posterior
probability distributions, point estimates, and credibility intervals for PMI, as
well as using the likelihood function to compute likelihood ratios for pairs of
hypotheses. Let us look further at the likelihood ratios for pairs of competing
hypotheses involving different intervals for PMI. A fictive case is used for
illustration of different scenarios where the main competing hypotheses are
the Prosecutor’s hypothesis \( H_1 \) and the Defendant’s hypothesis \( H_2 \) (as seen in
Paper IV, Table 2). If we have \( H_1 \): PMI of 7–9 days and \( H_2 \): PMI of 18–20
days we will have, more or less irrespective of the prior used for weighting
the hypotheses, a log_{10}likelihood ratio (log_{10}(LR)) for \( H_1 \) vs. \( H_2 \) near -0.61.
This means that the observed partial body scores are \( 10^{0.61} = 4.1 \) times more
likely under \( H_2 \) than under \( H_1 \). If we instead have hypotheses spanning longer
time intervals the log_{10}(LR) will depend more on the chosen prior used for
weighting the result. For example, \( H_1 \): PMI of 5–14 days and \( H_2 \): PMI of 14–
60 days. The highest log_{10}(LR) is obtained with the exponential discovery
prior and flat prior on a log scale, which places most weight on the shortest
PMI, within the timespan of \( H_1 \). If the discrepancy between the two competing
hypotheses is larger, the evidence will be stronger. An example could be \( H_1 \):
PMI of 5–7 days and \( H_2 \): PMI of 15–20 days. In this case, we will get a
log_{10}(LR) of between -1.04 and -1.06, depending on the prior chosen. This
could be translated into a semantic probability such as: “to a certain extent,
the findings support that \( H_2 \) is true, rather than \( H_1 \).”

Accounting for uncertainties in the training data

For cases within the training dataset with uncertainty in PMI, a highest and
lowest value of PMI was assigned to each case, based on the information avail-
able in police reports. The uncertainty consisted of the possibility that the de-
ceased was alive for some time after the last sign of life (e.g., when last seen
alive, newspapers or other contents of a mailbox, phone calls, etc.). The main
model (i.e., applying the EM algorithm for training data) allowed the uncer-
tainty concerning the PMI to be taken into account, and systematic differences
in the predicted $\log_{10}$ADD and SD were seen when comparing with the alternative models. The effect was most prominent when comparing the EM model with a flat linear prior, based on the assumption that all values between PMI minimum and PMI maximum are equally likely, with the ML model, where the upper bound for PMI was used as point estimate (Paper IV, Table 1 and Appendix figure S1). The upper bound for PMI correspond to the point estimate used in the original model in Paper I.

Performance of the model

The model’s performance was tested as a leave-one-out cross-validation. To measure of the model’s performance, we used the proportion of cases with true PMI within various prediction intervals. Using a flat prior on log scale, the proportions of cases within the 90% and 50% prediction intervals were 88% and 51%, respectively. Applying an exponential prior assuming constant detection probability ($p = 0.5$), the figures were 81% and 44%, respectively. Lastly, the flat linear prior resulted in 81% and 46%, respectively, of the true PMIs being within the corresponding intervals.
Discussion

Quantification of decompositional changes may have in several practical consequences. It may be a valuable source of knowledge and extending our understanding of the decompositional process and the many intrinsic and extrinsic factors affecting the rate and pattern in different conditions (e.g., environments). Moreover, it could also be a beneficial tool in PMI estimation, and allow possible differentiation between decompositional and pathologically or traumatically induced changes, hence improving the quality of the forensic investigation. In general, PMI estimation becomes more intricate as the degree of decomposition of the human body proceeds, when time has allowed the progression of various post-mortem processes, as well as the impact of several environmental factors. For this reason, established and reliable methods for estimation of long PMIs are limited [Knight and Saukko 2004].

This thesis comprises four papers investigating different aspects of the decompositional process of human remains found in an indoor setting, focusing on development of methods to improve the precision of PMI estimates. The results of the studies indicate that it may be possible to overcome some of the current limitations. The caveat of the established TBS/ADD method of Megyesi et al. [2005] is that the standard error of ± 388.16 ADD given by the regression equation formula results in a large uncertainty for short PMIs. On the other hand, this range of ± 388.16 ADD may be too narrow or unrealistic for extended PMIs (> 100 days). This problem of precision was also pointed out by Suckling et al. [2015]. The Megyesi study also included an incorrect linear regression analysis using ADD as a function of TBS. This would make the model completely dependent on the composition of the study population (i.e., training sample). For example, if we have many training cases with low ADD (short PMIs), a new case with a certain TBS may be attributed to a lower ADD than if you apply a model where many training cases have a high ADD (extended PMIs). The selection of cases gets an extremely large effect on the model. Moreover, decomposition is dependent on temperature and time (expressed as ADD), not the other way around. Moffatt et al. [2016] made several improvements to the statistical model used by Megyesi et al. [2005] and corrected the regression analysis (i.e., placed TBS correctly as a function of ADD). However, the small number of cases selected by Moffatt (15 of Megyesi’s 68 original cases) is a general weakness, making the model parameters, including the standard deviation, extremely sensitive to the sample and
creating a risk of introducing bias. This means that the standard deviation may not give a reliable idea of the PMI uncertainty in a new case.

The research presented in this thesis started with an evaluation of the TBS/ADD method in an indoor setting (Paper I). Due to the facts stated above, neither Megyesi’s nor Moffatt’s equations were used. The statistical model was carried out in a similar fashion as in Moffatt et al. [2016], but using the available taphonomic data from our cases. When applying the TBS/ADD method on our indoor forensic autopsy cases, several possible ways to further improve decomposition assessment and PMI estimation became apparent. Examples include modification of the TBS system to better reflect the decomposition in an indoor setting, development of novel scoring methods as a complement to TBS, and a new approach to the statistical modelling for better precision and assessment of uncertainties. Papers II–IV are the results of this ambition.

Paper I

The original purpose of the indoor decomposition project was to determine whether the TBS system could be used in our forensic autopsy cases, as well as to document the observed decompositional changes of human remains. As described in the Materials and methods section, a protocol with body charts (see Figure 1, p. 28) was set up and the human remains were assessed and scored directly at autopsy. The TBS/ADD method was adapted to our specific conditions with a focus on ensuring that the method was easy to use and could be a practical tool for assessing the degree of decomposition at autopsy. Additionally, it could be used to help the police to establish a timeline, when this was requested. We noticed that the descriptions for the partial body scores did not include some post-mortem traits that we had observed in our dataset. For example, head/neck (PBSH) marbling was visible on the face and scalp in some cases. Therefore, we also collected information about decompositional changes not included in the TBS system. One goal was to create a TBS indoor scale, in a similar way as the TBS has been modified for submerged or burned bodies [Heaton et al. 2010, Gruentahl et al. 2012]. However, this turned out to be a larger task, falling outside the scope of this thesis. A larger number of indoor cases would be needed, due to the diversity of decompositional changes that the indoor cases exhibited. Furthermore, in our sample of indoor cases, it was not possible to follow the progression of decomposition in the same body. Hence, a longitudinal study could be of value as a complement.

Moist decomposition and desiccation

Moist decomposition and desiccation seemed to coexist in this study sample, as well as various combinations of the two. It was apparent in some human
remains that the body position after death could lead to an uneven distribution of the decompositional changes, such as moist decomposition of the dorsal parts and desiccation of the ventral parts. The area of the body in contact with the floor often displayed moist decomposition, while the exposed parts could desiccate. The presence of insect activity was often associated with moist decomposition, but was also seen in combination with desiccation. We suspect that those cases started with moist decomposition and that the remains dried after the insect larvae consumed the soft tissue. Desiccation creates a hard shell of skin not appealing to insects [Haskell et al. 1997]. As regards the cases displaying desiccation in an indoor setting, it became apparent that several different processes must be involved, for example, in the development of the yellow to orange discoloration and translucent and parchment-like skin. It might be that this is an effect of decomposition of the subcutaneous fat, and that fat-rich fluids leak out through the skin creating these changes. There seemed to be an association to areas with larger amount of subcutaneous fat. These changes were often seen in anatomical regions usually containing thicker layers of subcutaneous fat, especially in cases with a higher BMI (i.e., obesity). This was in contrast to underweight individuals, who seemed to be more prone to desiccation after death, developing a darker brown colouration and a leather-like skin, as well as drying of soft tissues and internal organs.

In the method of Megyesi et al. [2005], mummification was considered one of the last stages of decomposition (i.e., moist decomposition had a lower score than mummification). This would mean that every other stage of the decompositional process, if the process is actually sequential, as suggested, had already occurred. In this study, we considered desiccation to be a process, while mummification represents a decomposition state, in analogy with Schotsman et al. [2011], stating that desiccation refers to moisture lost in tissue, while mummification refers to the point at which the decompositional process is halted. A body may never reach the last stages of decomposition, since it can be preserved and stay in this mummified stage for many years. The ADD method does not take mummification into account, since ADD will continue to increase even if the body does not reach the skeletonization stage [Parsons 2009]. Desiccation is a rather sparsely studied process, especially in a forensic context, with regard to PMI estimation.

Presence of insect activity

In this study, a significant seasonal difference was indicated, probably due to the presence of insect activity mainly during summer. The cases infested with insects were a rather heterogenous group and quantification of insect activity might improve the assessment of these cases. The dataset was compared with the original dataset of Megyesi et al. [2005]. All of the cases in Megyesi’s study were infested with insect larvae, probably to varying degrees. Only a few of our cases had an “outdoor characteristic” similar to what was seen in
Megyesi’s study. The restriction of insect access in an indoor setting may be one of the reasons that the precision of TBS and ADD in our model setup was lower. Without insect activity, the expected massive soft tissue loss is not seen. Moreover, in the cases that had presence of insect activity, this was unevenly distributed and only affecting parts of the body (e.g., head, neck, upper torso).

Generally, there is a risk for underestimation of PMI in cases with extensive desiccation and overestimation in cases with prominent insect activity (see Figure 2). However, it seems to improve when cases are divided into defined subgroups. The best fit of the model was found in the subgroup of cases without presence of insects, discovered during the fall and winter months. Those cases are probably more similar to each other, while cases discovered during spring and summer had a variable extent of insect activity, resulting in a wider range of TBS at similar PMIs within this group.

Indoor environment results in unique conditions

TBS is thought of as a value representing the accumulated decomposition in a dead body. However, this may not be the entire truth concerning the decompositional process in an indoor setting. We could see moist decomposition and desiccation as two separate pathways, rather than as a sequence. Some dead bodies may be prone to follow one of these pathways due to certain circumstances and specific factors affecting the course of decomposition. An example could be an underweight person, recently having undergone treatment with chemotherapy, dying in a dry apartment at an indoor temperature of +22 °C. This is a fairly hostile environment for bacteria, hampering any large spread of bacteria after death. The dead body desiccates. A counterexample would be an overweight person taking an overdose of drugs (which may cause a rise in body temperature before death [Zhou and Byard 2011]) dying in a humid apartment, also at an indoor temperature of +22 °C. This could be a perfect condition for an exponential growth of bacteria, resulting in putrefactive changes taking over; the pathway would lead to moist decomposition. These are two hypothetical situations. However, the ambient indoor temperature may not be a dominant factor in the decomposition in those settings. The existing microbial community in a body is likely to play an important role in the decompositional process, as are various other factors affecting it (e.g., fluid content in the body). Several recent studies have investigated the microbial succession in a dead body and considered a possible link to PMI [e.g., Metcalf et al. 2013, Finley et al. 2015, Javan et al. 2019].
Comparison with other indoor studies

When comparing our indoor study with the few other studies carried out, there are some similarities and some obvious differences in the PMI method performance, even if the same method was not used in all (i.e., the TBS system was not used). Vass’ [2010] universal PMI formula was tested in an indoor setting. When the observed soft tissue mass loss was more than 20% and PMI ≥ 4 days, the PMI estimate was found to be inaccurate [Maile et al. 2017]. In our study, insect activity was the main reason for soft tissue mass loss and in cases with extensive soft tissue mass loss, PMI was probably overestimated. In Vass’ [2010] universal formula, the decomposition represented by the soft tissue mass loss proved to be difficult to estimate and present as a percentage. Therefore, each decomposition stage (fresh, bloat, active decay, and advance decay) was associated with a percentage interval of soft tissue mass loss, for example bloat, 5–10%, and active decay, 10–50% [Maile et al. 2017]. In our Swedish indoor setting, with little insect activity, the soft tissue mass loss was not found to be extensive or comparable with these percentages. However, we did observe an association between increasing TBS values and decreasing post-mortem BMI considered to be an indirect sign of soft tissue mass loss. Gelderman et al. [2018] stated that PMI could be predicted in 67% of the indoor cases using their total decomposition score (TDS) method. However, in their study, we found the same statistical flaw as in Megyesi’s study [2005]. Therefore, a direct comparison with our study would be problematic. Hayman and Oxenham’s [2017] total body decomposition score (also called TBS, despite using other definitions than Megyesi’s TBS) is stated to accurately estimate PMI within several hours to half a day of death, in most instances, up to PMI of 14 days. This level of precision is not possible to achieve with our indoor method. However, their linear regression model was similar to that of Gelderman, meaning that questions can be raised about the statistical robustness of Hayman and Oxenham’s method. Further studies from these authors would be of interest.

All three indoor studies [Maile et al. 2017, Gelderman et al. 2018, Hayman and Oxenham 2017] indicate increasing difficulties in precisely estimating the PMI the longer the decompositional process has progressed. In our study, this might be a reason why PMI in cases with extensive desiccation appeared to be underestimated.

Statistical considerations

Moffatt et al. [2016] presented an improved equation for TBS and ADD, and pointed out some statistical flaws in the original study by Megyesi et al. [2005]. In this latter study, the authors had switched the explanatory (independent) and response (dependent) variables, resulting in an incorrect model, wrongly assuming that ADD could be predicted directly from TBS. This
“backward” regression model that Megyesi et al. [2005] and some other researchers used is principally incorrect and should be avoided, as Moffatt et al. [2016] pointed out.

The decompositional process, as represented by TBS, is the result of time and temperature (i.e., log$_{10}$ADD) in the model. Moffatt et al. [2016] uses an inverse prediction formula. When using this model to make conclusions about PMI or log$_{10}$ADD, given a specific TBS value, we have to make assumptions about the population from which the new case is drawn. If we assume any value of PMI is equally likely, we could use the formula proposed by Moffatt et al. [2016]. A principal weakness of the method of Moffatt et al. [2016] occurs when inversing the model, when we have to make rather strong and uncertain assumptions about the distribution of PMI within the population. An option would be using a Bayesian approach, as presented in Paper IV.

A Box-Cox transformation of TBS did not improve the fit of the model within our study’s limited ADD range. The original dataset in Megyesi et al. [2005] had higher TBS and ADD values (i.e., a wider range) and the transformation aimed to get a good fit across the entire scale. However, extrapolating for data with high TBS and/or ADD, the value of the exponent might have significant weight in a model, creating the risk that the result will no longer be reliable. This would be particularly relevant for small datasets, like that of Moffatt, consisting of only 15 datapoints (from Megyesi’s original 68 cases). In our dataset, we might have needed more cases with TBS < 5 and > 15 (i.e., expanding our range), to obtain a more reliable regression model. However, it cannot be excluded that an optimal regression model should be divided into different ranges, for example short PMIs, moderate PMIs, and extended PMIs.

Paper II

A few studies on sequential decomposition of human livers have been carried out previously, evaluating histological changes and their potential association to PMI [Kushwaha et al. 2009, Karadžić et al. 2010, Verma et al. 2015]. All of them focused on the first few days after death. The changes described seemed to evolve early, during the first day after death [Kushwaha et al. 2009, Karadžić et al. 2010, Verma et al. 2015]. In our dataset, the decompositional changes were seen slightly later; the majority of cases with PMI less than 1 day were without histological changes noticeable under a light microscope.

The results on the reliability of scoring liver slides in accordance with the HDS system indicated good reproducibility and consistent scoring.

The results regarding a Box-Cox transformation indicated that the optimal exponent in the training dataset ranged between 1.9 and 2.7 for the different HDS markers. The fitted model was applied in the independent validation dataset with equivalent performance as on the training dataset. The model with HDS markers had lower precision than the model based on the partial body
scores. However, the model combining the HDS markers and the partial body scores had slightly better precision than the models based only on the HDS markers or only on the partial body scores.

A possible way to improve the precision of the model might be to exclude some of the extreme outliers. This might result in a narrower confidence interval and smaller SD, i.e., better predictions. However, an objective criterion for identification of these outliers is needed to justify the exclusion. Decomposition is a complex process, in which many different factors play a role. Identifying these factors and to what extent they affect indoor decomposition is of importance for further improving the potential to achieve better PMI estimates.

The strength of the model is that the multivariate regression model utilises separate and correlated partial body scores, as well as the relatively independent HDS markers, to improve precision. This probably results in a better modelling of the decompositional process in a specific case, yielding an accurate and precise estimate of PMI. The model can be used in practical casework in a Bayesian framework for interpretations and for reporting PMI estimates.

Development and methodological considerations

In this study, the ambition was to find a practical way to assess internal decomposition, as well as to develop better methods for PMI estimation in decomposed human remains. The external decomposition has been observed to be uneven in some indoor cases (Paper I). Indoor cases may be more subjected to intrinsic factors due to the absence of weather changes such as wind, rain, temperature fluctuations, etc., as well as little or no animal scavenging and insect activity. The intrinsic factors affecting the rate of decomposition may thus be more important in an indoor setting than extrinsic factors, unlike for human remains decomposing in outdoor environments. The TBS system describes only the external decompositional changes. Our hypothesis is that an improvement would be possible internal decompositional changes could be assessed at the same time. This would provide higher resolution in the assessment of decompositional changes, thus resulting in the potential to increase the accuracy and precision in PMI estimates. The results of our study support this. A histological assessment method is a valuable complement to the TBS method. The liver has a well-defined histology and is situated in a rather protected position within the abdomen. We may assume that the liver is less exposed to some extrinsic factors than, for example, the skin.

Histological changes in the liver

The scoring method is based on well-defined structures in the liver tissue and an ability to grade them consistently. The identifiable structures, such as hepatocyte nuclei, will change over the course of time, i.e., providing a link to
PMI of the actual case. The histological structures of the liver do not decompose at the same pace; generally parenchyma cells may disintegrate faster than the collagen stroma. Hence, the separation of hepatocytes, portal area, and architecture were scored separately in the HDS system. Livers with extensive steatosis and/or cirrhosis were excluded since these conditions caused severe disturbances of the architecture [Lefkowitch 2015], meaning that a proper evaluation could not be performed. In the dataset used in our study, two cases with chronic inflammation in the liver were also present. The overall effect of this was not further explored. Ante-mortem changes to the liver, such as necrosis, for example due to acute intoxication or long-term use of liver toxic substances, e.g., paracetamol, would also be of interest to examine further. Interpretation of the cause of death will be dependent on the experience of the forensic pathologist to identify and distinguish pathological changes from decomposition. A histological quantification method is also affected by scoring (which is a subjective assessment, to some extent), processing, and staining the liver samples. Assessing the liver tissue samples and scoring in accordance with the HDS method requires some training. When applying the method to a new independent material, calibration between observers would also be beneficial.

**Upper PMI limit of the method**

In this model, the upper limit of the HDS was around a PMI of 30 days. Still, some cases with longer PMIs, e.g., one case with a PMI of 217 days, had assessable liver tissue not reaching the maximum score. In the higher score range of the HDS markers, collagen is still distinguishable. The exterior of the liver was macroscopically identifiable at the autopsy in spite of only collagen being visible at the histological examination. Characteristic for the cases with longer PMIs (> 30 days) was external signs of desiccation. Loss of water content (dehydration) probably affects not only the skin (exterior of body), but also the internal organs. The model’s performance was investigated by dividing the 236 cases into two groups: cases with or without external desiccation. A direct comparison between the two groups proved to be problematic due to a large difference in ADD between the two groups. Desiccated cases seemed to decompose at a slower rate and generally yielded a higher ADD.

Expanding the scoring method for longer PMIs (> 30 days) could be possible using other histological stains. However, a limitation of our study was that cases in the advanced decomposition stage were often devoid of liver tissue sample. Since the study was retrospective in nature, it was not possible to obtain more cases with higher TBS, to achieve a better range of decompositional changes within the dataset. A possible way forward might be a prospective study, as well as specific stains for collagen and a further examination of the decompositional process in extended PMIs. In the pilot study, a small sample of liver tissue in paraffin blocks from selected cases were processed and
stained with Gordon & Sweet’s silver stain (GS) for detection of reticular fibres (collagen type III). The GS clearly showed the alignment of hepatocytes and gave a good visualisation of the liver architecture. Differences in quality of the reticulin fibres were noted between cases with varying PMI. Since the dataset included very few cases in advanced decomposition stages, i.e., with heavily degraded liver tissue, it was not possible to fully assess the usability of GS stain within this study.

Paper III

Decomposition represents the progressive degradation of soft tissue altering the composition of carbohydrate, protein, and fat, creating favourable conditions for continuous microbial growth. This mixture organic compounds serves as a substrate for microbial production of ethanol and other volatiles detectable in the routine forensic toxicology analysis. Can microbial neoformation be associated with the degree of decomposition and/or the PMI? To answer this question, we had to take into account multiple variables in a highly complex biochemical processes, as well as the inter-variability between individual human bodies. A microbial succession takes place during the progression of decomposition. Recent studies have indicated the existence of a microbial clock, which can be used in PMI estimation with rather promising results [Finley et al. 2015]. If the favourable environment inside the decomposed body changes over time, along with the availability of substrates and the presence of different microorganisms, microbial neoformation might be a useful indicator and an aid in PMI estimation of decomposed human remains.

Microbial neoformation of volatiles

N-propanol and/or 1-butanol have been suggested as indicators of post-mortem ethanol neoformation in several other studies [O’Neal and Poklis 1996, Ziavrou et al. 2005, Ehrlich et al. 2010]. In our dataset, the occurrence of neoformation (i.e., presence of N-propanol and/or 1-butanol in femoral vein blood sample) was 42% in decomposed bodies, higher than the previously reported occurrence of 18–22% [Zumwalt et al. 1983, Gilliland and Bost 1993]. Moreover, in non-decomposed cases, the occurrence of neoformation was 3%. It is possible that the detected volatiles are produced during internal decompositional changes in the dead body, i.e., before any external signs are visible. The findings of neoformated ethanol in human remains without apparent external signs has been described in another study [Boumba et al. 2019]. However, to make a comparison between our study and the published studies turns out to be rather difficult. This is mainly due to the different ways of determining the occurrence of neoformation. Furthermore, the focus of most published
research papers is investigating whether the origin of ethanol can be determined, i.e., if post-mortem neoformation can be ruled out in forensic casework. The focus of our study, however, was if microbial neoformation could be used as a tool or objective measurement in PMI estimation.

During the decompositional process, the microbial activity may produce acetaldehyde [Corry 1978]. Acetaldehyde could therefore be an indicator of decomposition. However, it has been implied that acetaldehyde is a possible artefact [Boumba et al. 2012] and the presence of acetaldehyde varies widely between different studies [Boumba et al. 2012, Vezzoli et al. 2015]. Acetaldehyde occurred in 83% of the complete dataset in our study. Cases with neoformation had higher relative amounts of acetaldehyde than non-neoformation ones.

The dataset in our study consisted of forensic autopsy cases reflecting the common profile at our Department of Forensic Medicine. The majority of the decomposed cases exhibited changes representing early decomposition and bloating stage. The results indicate that neoformation cases (i.e., detected N-propanol and/or 1-butanol) had a somewhat higher degree of decomposition compared with cases without these volatiles detected. This means a higher TBS than expected at the same ADD in the neoformation cases. Most of the decomposed cases had a short PMI, though extended PMIs (one case with PMI 106 days) were present in our dataset. This profile of both degree of decomposition/TBS and PMI range may explain the results indicating a weak linear relationship between microbial neoformation and PMI and/or TBS. It is possible that a linear relationship exists within a specific range of PMIs or TBS, linked to the status of decomposition. Our dataset probably contained too few cases with bloating and active decomposition to further elucidate this possibility.

A novel way to improve the precision of PMI estimation

In this study, we presented the hypothesis that microbial neoformation of volatiles (i.e., N-propanol, 1-butanol, ethanol, and acetaldehyde) may act as an indicator of the decomposition rate. During early changes (within the first days after death), there is probably microbial neoformation. If this holds true, we would expect to see an increase in the amounts of volatiles due to abundance of substrate. After peaking, the amounts will decrease gradually over time. This decrease may be caused by elimination due to microorganisms using for example ethanol as an energy source [Corry 1978], but also a decrease in availability of the substrates needed for production of volatiles. The four volatiles investigated may have similar trajectories. We assume that at some point later during decomposition, there will be no difference in the decomposition rate between neoformation and non-neoformation cases. Femoral vein blood will also be gradually harder to retrieve from a decomposed body, i.e., an extended PMI creates a natural upper limit for the method. It may be feasible to
collect other body fluids instead, but further evaluation is required to determine if there are differences in amounts of volatiles between these other body fluids (e.g., cardiac blood or urine) and femoral vein blood. We believe that improvement of TBS/ADD method using the presence of volatiles would be optimal during the early decomposition stage and during the bloating stage. The microbial activity in the dead body during these stages is likely most favourable for volatile production.

The result of this study suggested that N-propanol and 1-butanol had the largest potential of the four assessed volatiles for PMI estimation. The amount of detected ethanol can be affected by ante-mortem intake of ethanol. If we could assess the origin of ethanol with certainty, this would be quite useful in our proposed method. It may be possible to use the two ethanol metabolites, ethyl glucuronide and ethyl sulphate, as indicators of ante-mortem intake of ethanol [Krabseth et al. 2014] to strengthen the assessment of ethanol-positive decomposed human remains. Vezzoli et al. [2015] used ethyl glucuronide in combination with N-propanol and acetaldehyde (as indicators of neofor- mation) when assessing post-mortem femoral vein and heart blood samples. However, the degree of decomposition of each autopsy case positive for ethanol determined to be of ante-mortem or post-mortem origin, respectively, was not given in this study. Ethyl glucuronide can be degraded during the decomposition of the body [Høiseth et al. 2008]. Ethyl sulphate may be more stable [Baranowski et al. 2008] and can prove to be suitable to use in heavily decomposed bodies. When we began the study of microbial neoformation in our forensic autopsy cases, we were planning to retrieve additional blood samples for ethyl glucuronide and ethyl sulphate analysis to be used in combination with analysis of other alcohols and acetaldehyde. We also thought about collecting and analysing several different fluids such as heart blood, vitreous humour, and urine. The study presented in this thesis was later limited to a retrospective assessment of femoral vein blood samples.

The amount of acetaldehyde varies widely, although there is a tendency towards higher amounts in connection with neoformation (presence of N-propanol and/or 1-butanol) in cases with external decompositional changes. The importance of this volatile was therefore difficult to interpret.

By applying a linear regression model of TBS ∼ rate-modified log₁₀ADD (i.e., log₁₀ADD + log₁₀volatiles), we achieved a model with a better fit. This model could be applied to a new case when TBS and the relative amounts of volatiles are known. As presented in Paper I, there are some limitation of the TBS system in an indoor setting, and as Papers II and IV indicates, improvements can be made. Assessing volatiles (alcohols and acetaldehyde) in blood samples collected from human remains in an indoor setting points to a way forward towards a deeper understanding of the indoor decompositional process and towards opportunities to improve PMI estimation.
Paper IV

There could be substantial uncertainty in the estimation of PMI when only working with a scoring-based method such as TBS, HDS, or any other PMI method based on post-mortem changes. It would therefore be desirable to reduce this uncertainty and also make clear the level of uncertainty regarding a specific forensic case. In forensic investigations or in court, it would be of value if a PMI estimation based on decompositional process could be weighted correctly against information about PMI derived from other sources (e.g., evidence at the discovery/crime site, witness statements, etc.). The decompositional process per se may result in a large uncertainty due to biological and environmental factors affecting the process to varying extents. These factors may not always be possible to adjust for or are unknown at the time the PMI estimation is carried out. Still, even PMI methods with low precision can be useful in forensic casework [Madea et al. 2019]. It is equally important to know when the uncertainty is too large for an accurate and reliable estimation of PMI to be performed and communicate this during forensic investigation or in court.

In a specific case, the forensic evidence consists of the taphonomic data represented by the partial body scores observed during the examination of the dead body and used in PMI estimation. We can establish a stochastic model if we have cases where values for the partial body scores have been observed together, with corresponding values for PMI, by relating the two. Given a specific observation of the partial body scores in a new forensic autopsy case with an unknown PMI, this stochastic model can be used to compute the probability of observing these specific value of partial body scores for all possible values of PMI. This function of PMI is called the likelihood function. A confidence interval for log_{10}ADD and thus PMI may subsequently be computed, if we have information on the distribution of PMI in the population.

Bayesian framework for reporting taphonomic evidence

In this work, a novel Bayesian framework was constructed which can be used in practical forensic work for reporting results (i.e., the PMI estimate). The framework was implemented in a model for indoor cases without presence of insect activity, using the three partial body scores of head, trunk, and limbs (PBSH, PBST, and PBSL). It would then be possible to implement the fitted model of both partial body scores and HDS markers as presented in Paper II, as well as incorporating other information, for example concerning desiccation. We use prior information about PMI combined with the likelihood function to get a posterior probability distribution. We can use the likelihood function to compute the relative weight of evidence in relation to competing hypotheses. The prior information has to be derived from other sources than the taphonomic data, representing our prior beliefs concerning the time of death.
To understand the impact that our choice of approach has when reporting evidence, assume there were two conflicting hypotheses for the time of death; PMI of 60 days or PMI of 90 days. By using the Bayesian framework, the forensic evidence can be summarised as the likelihood ratio, i.e., the ratio between the likelihood function values for 60 and 90 days. These values are shown in Paper IV, Fig. 1. A computed likelihood ratio of 2.4 means that the forensic evidence gives some support to a PMI of 60 days rather than a PMI of 90 days, i.e., the likelihood is 2.4 times higher at 60 days than at 90 days. Using the scale of the Swedish National Forensic Institute (NFC), a likelihood ratio of 2.4 indicates very weak forensic evidence and is said to “speak neither against nor in favour of the prosecutor’s hypothesis”. In Paper IV, the box in the right upper corner in Fig. 1, we can see that the hypothesis of 90 days is outside the 95% confidence interval for PMI, whereas the hypothesis of 60 days is within the interval. In court, if only the confidence interval is reported, the court might decide that 90 days is excluded, and judge that 60 days is the only possibility in this case. If the court were instead presented with the result of the Bayesian approach, their conclusion might have been quite different.

Different priors and case scenarios

Choosing one prior is essential for the outcome of the model and how the results are interpreted. We presented four different plausible priors that could be described as follows 1) longer PMI is more likely (flat linear prior), 2) shorter PMI is more likely (flat prior on log scale), 3) the discovery of the dead body after a short time is more likely (exponential discovery prior) or 4) death occurring in close proximity to last being seen alive is more likely (exponential survival prior). Other priors could be defined. Conclusions made about PMI will depend on the prior used. However, we believe that a forensic report in real forensic casework must include the likelihood curve, as well as clear definitions of the different priors used. It is of importance that the forensic report is unbiased and maintains transparency. Certain approaches may be appropriate in some cases, but misleading in others.

Three different types of scenarios are suggested: (i) the blind case, (ii) the case with prior knowledge, and (iii) the case with competing hypotheses. One specific case may start off as a blind case and then evolve through the other case types at later stages.

When a decomposed human body is recovered, the identity of the deceased is in many cases not known (blind case). Reporting a PMI estimate could aid the identification, as well as providing the police investigation a possible timeframe within which the time of death may lie. The default assumption would be the flat linear prior, i.e., intervals of time of equal lengths are equally likely (resulting in longer PMIs being more likely). The posterior distribution obtained with this prior is equivalent to the frequentist confidence intervals proposed by Moffatt et al. [2016] who implicitly assumed that the case came
from a population with these characteristics. The majority of dead bodies are recovered after a short time and therefore have a short PMI. However, using the **flat linear prior** might risk overestimation of the PMI. If we instead use the **flat linear on a log scale prior**, which has a rather good fit with our training data, the probability of the case having a short PMI (e.g., 1–7 days) might be overestimated for a new case. A more realistic posterior distribution may be obtained by applying an **exponential prior** under the assumption that the probability that the dead body is recovered would be constant over time. However, the parameter of the **exponential prior** would depend on how exposed the finding site is and thus the time of detection of the dead body. For example, the odour from a decomposing body may alert the neighbours in an apartment complex.

In the case where the dead body has been identified and some information about the possible time when the person was last seen alive will be available (case with prior knowledge). This information could set the upper bound for PMI, and a **truncated prior** can be chosen. In contrast to the blind case, the short PMI may no longer be likely. In some cases, a **truncated flat linear prior** may be suitable, but in other cases it is more realistic to assume that the person died in close proximity to the time last seen alive. We may use an **exponential survival prior** or possible construct a case-specific prior. Both the **flat linear on log scale prior** and the **exponential discovery prior** may underestimate PMI.

A posterior probability distribution may be more suitable in a blind case, communicating a time interval to, for example, the police, while a likelihood ratio is more usable in a case with prior information and different hypotheses to be weighed.

When a dead body has been identified and prior knowledge is available, for example, in a suspected homicide, there may be conflicting statements and the different hypotheses concerning the time of death must be evaluated. The PMI estimate is often just one part of the evidence comprising a forensic investigation. In this situation, a point estimate or confidence interval for PMI may provide little guidance. The Bayesian framework provides the possibility to report the results as a likelihood ratio between conflicting testimonies, in accordance with the framework adopted by several European forensic institutes [Nordgaard et al. 2012]. This approach is directly computable when the conflicting hypotheses refer to two relative short intervals, such as “7th of June versus 14th of June”. Concerning decomposed human bodies, hypotheses with more extended intervals may be more reasonable. The decompositional process per se may give a relatively large uncertainty in PMI estimates based on taphonomic data. The important question raised by a court, for example, could be “time of death before or after 14th of June?” We have to consider the probability that evidence is not evenly distributed over the time intervals specified in the conflicting hypotheses. It would become necessary to define the upper and lower boundaries of the intervals and to compare and provide a weighting
function for the different possible values of PMI within the interval. We pre-
sented four different priors in our paper and would recommend trying out sev-
eral different priors as the weighting function. As we can see in Tables 1 and 2 in Paper IV, the values of LR obtained with different priors are often similar and would in many cases result in the same conclusion. However, if these values are very divergent, there is a risk of interfering hypotheses, making it difficult to separate one hypothesis from another. A possible reason for this might be that the available information about the circumstances in the specific case may be vague or lack important elements, resulting in difficulties in ob-
taining good assessments.

Methodological considerations

When the observed partial body scores provide more exact information about the time of death, the posterior distributions will be more similar, i.e., less influenced by the prior distribution. Due to this impact of the choice of prior, we recommend that care is taken to ensure that the chosen prior properly re-

flects the type of forensic casework and the circumstances in the situation. The likelihood function is objective and shows how likely it is to observe a com-

bination of the three partial body scores at different PMIs. If we want to cal-

culate the probability for a certain PMI (or time of death), we have to either make assumptions concerning the population from which the new case was or, when we use a Bayesian framework, select one or several priors. The first option is common in frequentist statistics, but it is perhaps not as obvious for the recipient what assumptions was made, while in the second option, with a Bayesian framework, a prior could be clearly specified.

Using our indoor dataset as a training data, there were some inherent un-
certainties affecting the predictions made based on the observed partial body score and ADD of each case. For example, the true PMI was estimated from witness statement, oldest newspaper at site etc., with a risk of overestimating PMI. The temperature at the discovery site was not known for every single day during the time interval, though indoor temperatures were contained within a limited temperature range. There could be a suboptimal fit of the partial body scores’ descriptions for indoor decomposition, not completely reflecting the degree of decomposition. The Bayesian approach is still useful, but the predictions made could be weaker in some cases (i.e., have larger uncertainty). It may be possible to use the HDS markers (Paper II) and microbial neoformation of alcohols (Paper III), or – when applicable – forensic entomol-
ogy to strengthen the PMI estimates in a specific case. The Bayesian frame-
work offers a logical and useful tool that may be expanded and refined de-
pending on the circumstances.
Conclusions

The main conclusions of this thesis are:

**Paper I:** The TBS method needs to be slightly modified to better reflect indoor human decomposition. Furthermore, it requires distinct inclusion criteria and a defined population.

**Paper II:** In practice, the HDS system offers a structural and systematic way to assess the degree of decomposition and has good potential as an aid in PMI estimation.

**Paper III:** Microbial neoformation of volatiles reflecting the rate of decomposition may be used in PMI estimation based on the TBS/ADD method, as a possible way to improve its precision.

**Paper IV:** The Bayesian approach to interpretation and reporting PMI estimates is a flexible system, adaptable to different priors, providing the possibility to define and handle uncertainties in a given investigation. It can be used in an individual case where applying assumptions at the population level is inappropriate.
The quest to improve the accuracy and precision of PMI estimation based on the decompositional process has been pursued by several researchers over the past 30 years [e.g., Galloway et al. 1989, Mann et al. 1990, Vass et al. 1992, Bass 1997, Megyesi et al. 2005]. The establishment of outdoor human forensic taphonomy facilities (commonly known as “body farms”) offering the possibility to study the decomposition of human bodies in detail has led to a rapid increase of research articles within this field [Varlet et al. 2020]. However, the indoor setting as a distinctive environment is not subject to the same extensive research. Finding a PMI estimator of high precision is somewhat of a holy grail. A deeper understanding of the biochemical mechanisms driving the decompositional process would be fundamental to refining methods for estimation of PMI. The rate of decomposition is an important part of this. It is suggested in several studies that clothes and coverings, body size, wounds, presence of insect activity, and animal scavenging, etc., may interfere with the rate of decomposition for the whole body or for a part of the body [e.g., Bass 1997, Steadman and Worne 2007, Matuszewski et al. 2014, Smith 2014]. The studies comprising this thesis have also indicated that desiccation, insect activity, and presence of neoformation of volatiles can cause clear changes in the rate of decomposition. Subdividing cases into groups based on these indications makes it possible to increase the precision of the PMI method. Perhaps a more individualised PMI estimator, taking into account the specific circumstances of a forensic case, could be a way forward.

In this thesis, a foundation for further development of methods for estimation of PMI in decomposed human remains found in an indoor setting is proposed. The TBS/ADD method was originally designed for field use. The advantages of TBS are that it is easy to learn and fast to use. Sampling liver tissue and femoral blood for further processing takes more time. In general, the forensic pathologist’s experience affects the consistency of scoring. The histological assessment takes somewhat longer to learn than using TBS. Despite this, the proposed methods in this thesis are faster and easier to use than, for example, analysis of DNA degradation or presence of different microorganisms. Femoral blood and tissues samples are routinely collected and available.

As stated in the introduction to this thesis, an important matter in forensic science is to have appropriate standards for interpreting forensic evidence (i.e., in this case, the data retrieved from decomposed indoor human remains) and
presenting evidence – with its inherent uncertainty – to police or to court. Introducing the concept of a Bayesian framework to the forensic medicine community may be challenging. I believe that it would be beneficial to use statistical models which enable us to compute a likelihood function for a given case, i.e., the relative probabilities of the observed data among many possible PMIs. Further, I have observed that there is a need to learn how to use for example a posterior probability distribution or a likelihood ratio to communicate results in a way that can be a reliable aid in a decision-making process. Not only how to communicate uncertainties associated with PMI estimates, but also to whom evidence is communicated, is of importance. A recipient has to comprehend and be able to interpret the information received. Establishing best practices for PMI estimation of decomposed human remains, as well as for communication of these estimates is greatly needed.
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Folkhälsomyndighetens allmänna råd om temperatur inomhus (FoHMFS 2014:17). [The public health agency of Sweden’s general advice on indoor temperature].


66


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)