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STUDY OF THE DIFFUSION OF HUMAN'S VOLATILE ORGANIC COMPOUNDS (VOCS) IN AVALANCHE SNOW: A POTENTIAL CHEMICAL TOOL TO IMPROVE TRAINING OF AVALANCHE DOGS.

STUDY OF THE DIFFUSION OF HUMAN'S VOLATILE ORGANIC COMPOUNDS (VOCS) IN AVALANCHE SNOW: A POTENTIAL CHEMICAL TOOL TO IMPROVE TRAINING OF AVALANCHE DOGS.

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..e, se nun entra nelle spese tue,
perch'è c'è un antro che ne magna due.
— Trilussa, *La statistica*

to men of good will

ABSTRACT

In recent decades the number of people that decide to spend their free time in mountain is constantly growing. In particular during the winter season, the human presence in the mountains enhances the avalanches hazard and increase the number of accidents. Between the categories at higher risk, as alpine skiers, off-piste skiers and hikers, there are also people performing activities for which it is not expected the use of avalanche transceivers. Therefore, if people buried in avalanche are not equipped with a locating device, their survival chance is linked to the speed of the organized rescue and to the efficiency of avalanche dogs.

Nowadays, despite the skills of the trainers and the proven ability of search dogs, there remains a considerable margin of improvement in training of avalanche dogs. Since is impossible to simulate the condition in which the people are buried in avalanche, it is not possible to train dogs in an environment similar to the real situation. Currently, trainers of avalanche dogs can simulate the buried body with a volunteer, but this experiment presents many difficulties, therefore are commonly used objects that have been in contact with the human body. However, it is not yet clear whether this method is effective to train dogs to search for buried people or if it is misleading. Moreover there isn't a certificated olfactory target.

The production and use of a certificated training kit for avalanche dogs, like for example the training kit for drug dogs, could be made only if the chemical substances emitted by the buried are known. Since the development of such olfactory target could represent a great achievement in the context of avalanche rescue and as the issue of the diffusion of organic compounds in the snow is an open field of investigation, we started to study the body odour to select appropriate molecular targets.

We developed a qualitative analytical method for the analysis of VOCs from sweat. Sweat was choose since dogs are currently trained with objects that had been in contact with the skin. We performed extraction of real sweat samples through headspace solid phase microextraction followed by gas chromatography - mass spectrometry for separation and identification. We were able to identify several compounds and we have selected among these 3-hydroxy-3-methylhexanoic acid, 3-methylhex-2-enoic acid and 3-methyl-3-sulfanilhexan-1-ol since they are specific of the human odour.

In order to provide a reliable tool for investigating diffusion processes of the specific components of the human odour through the snowpack we developed and optimized an analytical method based

on direct immersion solid phase microextraction followed by gas chromatography - mass spectrometry. Solid phase microextraction was performed using polyacrylate fibers placed in aqueous solutions containing the targets. We opt for direct immersion instead of headspace extraction because of the higher precision shown in our experiments. The analytical performances of the method were evaluated in terms of extraction efficiency, repeatability, reproducibility, linearity, limits of detection (LOD) and quantification (LOQ), accuracy and we demonstrated the applicability of the method to real cases of melted snow samples.

We designed an innovative methodology to simulate the processes of diffusion of molecular targets from the body within the snowpack. Body temperature was simulated with a heat source and a cotton pad soaked with artificial VOCs was used to mimic the presence of a buried body. The heat source toghether with the olfactory target were buried under 50 cm of snow from 20 up to 80 minutes, then snow was sampled at differents depth on the vertical of the olfctory target. Samples were kept frozen in vials and transported in laboratory for analysis. To guarantee a reproducible and standardized procedure we developed a core barrel whose innovative property is the ability to make available the inner snow sampled. To simulate the conditions of the avalanche snow, we prepared an artificial avalanche site where to perform the test. Snow properties such as temperature, density and type of grains were monitored during all the experiments to guarantee the same environmental conditions for all the tests.

The research was conducted in close collaboration with the Soccorso Alpino della Guardia di Finanza di Predazzo, with the Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto and the Laboratorio di Etologia e Benessere Animale at Department of Veterinary Medicine of Perugia University

Keywords: Body Odour, VOCs, HMHA, 3MSH, SPME, Avalanche dogs.

PHD PUBLICATIONS

[1] Federico Dallo, Dario Battistel, Rossano Piazza, Jacopo Gabrieli, Jean-Jacques Filippi, Nicolas Baldovini, and Carlo Barbante. "Direct immersion solid-phase microextraction with gas chromatography and mass spectrometry for the determination of specific biomarkers of human sweat in melted snow." In: *Journal of separation science* (2016).

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ACRONYMS

S.A.G.F. Soccorso Alpino della Guardia di Finanza

C.N.S.A.S. Corpo Nazionale del Soccorso Alpino e Speleologico

GdF Guardia di Finanza

C.I.S.A.-I.K.A.R. Associazione Internazionale del Soccorso Alpino

CAI Club Alpino Italiano

CSA Corpo di Soccorso Alpino

A.I.N.E.V.A. Associazione Interregionale NEve e VAlanghe

A.R.P.A.V. Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto

A.R.T.V.A. Apparecchio di Ricerca dei Travolti in VAlanga

U.C.V. Unità Cinofila di ricerca in Valanga

F Form of grain

E Size of grain

SSA Specific Surface Area

TSA Total Surface Area

DVB/CAR/PDMS Divinilbenzene/Carboxen/Polydimethylsiloxane

SPME Solid Phase MicroExtraction

VOCs Volatile Organic Compounds

GC-MS Gas Chromatography - Mass Spectrometry

CAS Chemical Abstract Service

HS HeadSpace

DI Direct Immersion

PTFE PolyTetraFluoroEthylene

Part I

INTRODUCTION

1

INTRODUCTION

Recent years have seen a rapid improvement in the techniques for the localization of people buried in avalanches, such the use of radio transmitters (transceivers). However, when avalanche victims are not equipped with transmitter equipment, the use of rescue dogs remains the most effective method for locating them.

In these rescue operations an important role is played by the organization of rescue team, the time of arrival of the rescuers at the avalanche site by helicopter and the time required for locating the buried person. In such cases, canine rescue teams such as the canine units of the Alpine Rescue of Guardia di Finanza are mobilised.

Nowadays, however, the procedures for training dogs do not follow a standard certificate protocol since it is not possible to train dogs in condition that are close to reality. The lack of a protocol analogous, for example, to the one standardised for anti-drug dogs, results in a heavy cost for collectivity since years of training do not guarantee the operational effectiveness of the rescue unit.

Although the level of training of rescue dogs is generally good, and Italian canine units are at the top of international rankings, there remains a considerable margin of improvement. Such an improvement could be achieved through innovative training methods based on the analysis of the performance of dogs trained in a novel multidisciplinary context that involve the use of molecular markers of human body odour.

Among the problems that make this kind of study difficult, there is our lack of knowledge about the chemicals emitted by the human and about the physical variables that govern the fate of volatile and semivolatile organic compounds in snow. Moreover, we still need to study a dog's olfactory ability to discriminate among the different classes of molecules emitted by the human body (sweat, breath, clothes, etc.) and how they reach the surface after moving through the snow mass.

This dissertation seeks therefore to shed light on this new field of research, made possible today by the development of modern instruments of analysis by the collaboration between academic research and the Guardia di Finanza. One of the ultimate goals of this study is to select specific olfactory targets that can be used for training avalanche dogs.

1.1 AVALANCHES

Avalanches are masses of snow, sometimes including rocks, earth and ice, falling from a mountainside. When avalanches occur, people are often injuried or killed and buildings and the environment are also damaged. There is therefore a deep interest in studying these natural phenomena and in developing techniques and procedure to protect people and things from their destructive power.

1.1.1 *Historical avalanches*

The term avalanche hazard refers to the involvement of people and to the destructive effects of avalanches. Such a risk is always high in mountain resorts and in valleys with high concentation of population, as in the Alps.

For example, in 1951, there were 98 deaths in Switzerland, 143 in Austria in 1954 and in both years several settlements were destroyed in different parts of the Alps.

One of the most disastrous avalanches in recent European history was the one that occurred in 1970 in Val d'Isere in the French Alps, when a hostel was destroyed and 39 people were killed. Also in 1970, on February 24, an avalanche swept over 6 buildings in Reckingen, Switzerland. 30 people died and 18 were rescued. In the same place a potentially similar disaster was avoided in 1978 thanks to the avalanche barriers.

In Gusikar, India, in 1979, an avalanche destroyed several buildings in stone and made 35 victims. During the same week, another 200 people died in the Himalayas[1].

More recently there have been also serious accidents. In 1999 in Austria, a detachment of snow slabs with a front 50 metres high hit the village of Galtür at 290 km/h, destroying cars, buildings and burying 57 people, 31 of which were found dead. Galtür was also an important testing ground for modern avalanche dogs. Among the rescuers we should remember several dogs that proved their worth on the field, working continuously for 24 hr searching for missing people. Let us mention in particular Heiko, a search dog, and Jack, a German Shepherd who was buried by the avalanche (bbc.co.uk).

1.1.2 *Socio-economic consequences*

The real social and economic costs linked to avalanches are hardly quantifiable as they remain largely hidden. However, many economic sectors are involved when an avalanche occurs.

Transports: Avalanches cause the interruption of traffic on roads and railways crossing the mountain valleys. This forces companies and administrations to bear the costs of snow removal and of the recon-

struction of infrastructure. Not to mention the other casualties often involved in these cases, such as wounded, dead and destroyed vehicles.

Buildings: avalanches destroy buildings and injure or kill the residents. This is why, in the localities at risk, engineers must carefully select the site, design secure buildings and protective barriers for homes, mine sites, phone lines, pylons and ski lifts.

Tourism: The death of people in avalanche accidents may compromise the reputation of resorts, discourage tourists and consequently greatly restrict the services offered.

Avalanches affect numerous other economic activities (such as forestry) and, globally, the life of mountain inhabitants. Moreover, as more and more people come to the mountains to practice sports and leisure activities, their encounters with avalanches increas as does the demand for security. This is why today considerable investments are made to secure homes, transport route and ski resorts, to keep up with the increasing traffic and to maintain a special army rescue corps.

The annual rate of avalanches that involved people is stable on average, although very variable from season to season. However, humans began to affect this frequency through their outdoor activities[1, 2].

1.1.3 *Mountain sports and tourism*

Since the seventies, mountain outdoor activities have been increasingly practiced, especially skiing, ski mountaineering, ski tours and mountaineering *tout court*. So, if before 1970 70-80% of avalanche accidents affected homes and roads, after 1970 90% of recorded avalanche victims were practicing sports and tourism activities.

This change of trend is on the one hand due to the increase of the barriers and works of prevention that secure buildings, and on the other hand is representative of the increasing number of tourists, often technically and culturally unprepared, motivated by a longing for wilderness and by increasingly cheaper equipment.

SOME STATISTICS: In the Alps, from 1960 to 1970, 2176 people died, 1070 of which were alpine skiers (49.2 %), 539 off-piste skiers (24.8 %), 261 mountaineers (12 %), 67 skiers on the ski run (3.1 %), 103 in roads (4.8 %), 51 in house (2.4 %) and the remaining 4 % in other categories that included leisure activities such as hunting and conductors of snowmobile. Thus only 7-8 % of avalanche victims were not practicing sports or entertainment[1]

From 1976 to 1995 2924 people died in the countries belonging to Associazione Internazionale del Soccorso Alpino (C.I.S.A.-I.K.A.R.), an international organization whose members include Andorra, Argentina, Australia, Austria, Bosnia and Herzegovina, Bulgaria, Canada, China, Croatia, Czech Republic, France, Germany , Greece, Iceland,

Ireland, Italy, Japan, Liechtenstein, Macedonia, Montenegro, Nepal, New Zealand, Norway, Poland, Romania, Scotland, Serbia, Slovakia, Slovenia, South Africa, Spain, Sweden, Switzerland, United Kingdom and the USA.

In Italy during the same period 461 people died, an average of 23 per year. A study of the Associazione Interregionale NEve e VAlanghe (A.I.N.E.V.A.) (web page), an institution that also deals with statistics in Italy, estimated that in 1987-1995 the avalanche deaths were 185, including 86 alpine skiers (46.5%), 31 off-piste skiers (16.8%), 14 on-track skiers (7.6%), 40 climbers (21.7%), 14 in different categories (7.6%), and zero in the streets or in homes.

Finally the Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto (A.R.P.A.V.) studied the period from 1967 to 2009 counting 827 deaths with an average of 19 victims per year over that period, which decreases to 17 deaths per year if we take into account only the last 10 years of the study. From 1984 to 2009 it is estimated that 2032 people were involved in 954 avalanches, 466 (23%) of which died and 1566 (77%) survived[2]. Similar percentages were also observed in Switzerland[3] and France[4]. Of these victims, 50% practiced off-piste skiing, 19% climbing, 17% freeride, 3% were in-area skiers, 2% on the road, 1% in buildings and 9% other categories (such as walking with snowshoes, an activity that made 10 victims from 2005 to 2009). As a summary, the following statements can be made[1]:

- During the last twenty years (1980-2000) about 2200 people died in the Alps. If we consider that the average number of victims involved in avalanches is four of wich one dies, one is wounded and two are uninjured, the number of people involved is about 9000;
- the annual number of victims varies considerably, as it depends on the relationship between the nivo-meteorological conditions and the periods of attendance of mountains (weekends, holidays);
- the majority of the victims are ski mountaineers and backcountry skiers (about 80%);
- over 90% of the victims practiced sports and leisure activities;
- statistics in Italy, France, Austria and Switzerland are similar;
- avalanche barriers and prevention work have virtually eliminated deaths in homes;
- the improvement of professional skills and technologies available to the rescuers have substantially increased the chances of survival of those buried in the avalanche.

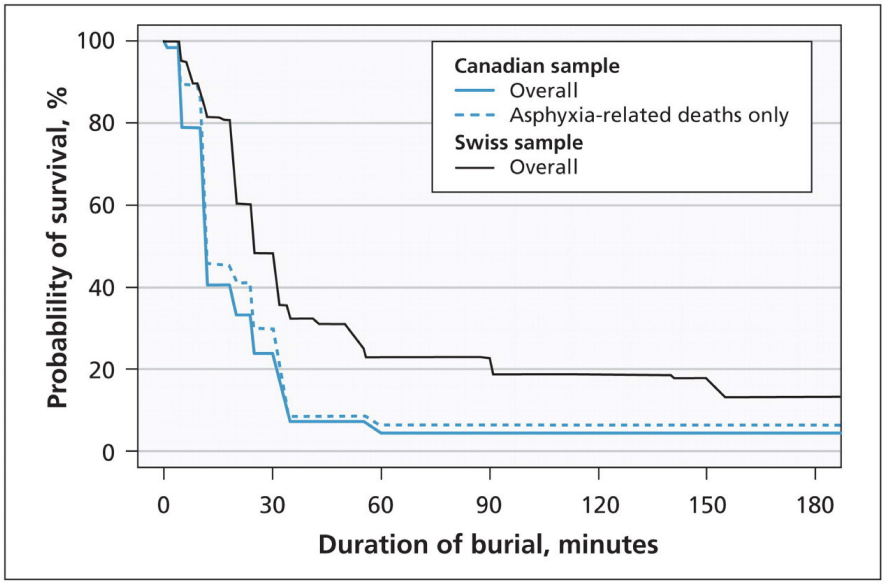


Figure 1: Overall survival curves for people completely buried in avalanches in Canada (n = 301) and Switzerland (n = 946) from Oct. 1, 1980, to Sept. 30, 2005, by duration of burial (Dümbgen comparison: p = 0.001). The dotted line represents the Canadian survival curve including only asphyxia-related deaths (n = 255)[5].

1.1.4 *Survival chances*

The impact of an avalanche can kill instantly. If the victim survives, death occurs from lack of oxygen. This can happen in a few minutes but in some cases the metabolism slow down because of the cold, thereby extending their survival by several hours.

The probability of survival depends on the burial depth, the nature of the avalanche and the way in which the victim is trapped. Moreover, the probability of finding a person buried alive decreases rapidly with time. On average only 40% of those buried are found alive.

In general the probability of surviving increases if the buried person is close to the surface, where the snow is less dense. Recoveries of people alive after being buried in more than 2 metres deep are rare (4%). Indeed the pressure of the snow exerted on the body prevents the buried person from breathing, leading to the formation of an ice mask in front of the face and finally to freezing.

Recent studies by Brugger, Falk and Haegeli on the survival of avalanche victims indicate that the probability of avalanche survival is 43%[5–7]. However, the most important observation is that the death rate when the intervention of organized rescue is needed is 86%, while in the case of a partner's rescue the lethality is around 32%. This is clearly due to the time required by rescue teams to reach the avalanche site.

The survival curve (Figure 1) indicates that 92% of buried victims are still alive for 15 minutes after a burial (*stage of survival*), while 8% died just after the accident because of injuries and trauma. Between 15 and 35 minutes after the burial there is a sharp fall in the chances of survival, from 92% to 30% (*asphyxiation phase*). In practice the buried victim who does not have an air pocket inside the snowpack dies because of suffocation. It is at this stage that the majority of buried die (65%). If the buried survive this stage, statistically 27%, can survive up to 90 minutes (*latency phase*). After this phase almost nobody survives and the victims die between 90 and 130 minutes. This because after about 90 minutes the critical body temperature of 32 °C is reached (the hypothesis is that in these conditions a person loses about 3 °C/hr). Oxygen is also lost in favor of carbon dioxide. Only in fortunate cases (3%) the buried can breathe fresh air, and has a chance to survive beyond 130 minutes.

It is possible to draw the following conclusions from the analysis of the survival curve:

- The victim's companion must try to recover the buried within 15 minutes of burial, since it is at this stage that a person has the greatest chances to be saved (mean Italian time is 10 min[8]). This explains the great importance of awareness, preparedness and use by the mountaineers of transceiver equipment for rescue (Apparecchio di Ricerca dei Travolti in VAlanga (A.R.T.V.A.)), shovel and probe[9, 10].

- Organised rescue teams must be able to reach the place in a time not exceeding 90 minutes (mean Italian time is 62 min[8]).

The organization of the rescue must therefore be very efficient and at least the canine units have to be ready to depart quickly after the call[11, 12], also because the time needed by a dog to discover a buried victim is about 10-15 minutes[13].

Another interesting analysis relates the burial time with depth. For a person buried at a depth up to 50 cm, the average recovery time is 10 minutes, from 50 cm to 1 m the average recovery time exceeds 50 minutes, and for a buried below 1 m the recovery time exceeds 2 hours. Thus, 77% of those buried under less than 50 cm survive, 33% within 1 m and only 19%[1] below 1 m.

1.1.5 *Monitoring authority*

In recent decades there has been a considerable development in the study of prediction and prevention of avalanches in Italy. Historically the first initiative in the systematic study of avalanche was carried out in 1956 when the Inspectorate of Forestry of Sondrio sent several mountain guides to Davos to specialise on the observation of snow conditions and avalanches. Later was the foundation of the Snow and

Avalanche Center Observation of Bormio in the late 1950s and in 1960 there was the first course in Italy for Osservatori Neve e Valanghe.

In 1967 Commission of Snow and Avalanche, later renamed Avalanche Service, was formed within the Scientific Committee of CAI. The Commission organised the first weather stations in the Alps, about 100 in 1975. Then, in collaboration with Davos, the Commission has produced the first avalanche bulletins and today it operates inside the CAI with disclosure and prevention purposes (http://www.cai-svi.it/).

At the end of 1970 the responsability for the study and management of avalanche prevention was given to Italian regions. Each region was equipped with its own office for the study of snow and avalanches, but to ensure uniformity A.I.N.E.V.A. was founded in the early 1980s.

The association aims to ensure the coordination of forecasting and safety actions and common initiatives such as the methodology for the study of snow, avalanche and mountain meteorology. The association also aims to promote the exchange of information between offices and encourages the adoption of uniform means and tools for monitoring and data processing. Finally, A.I.N.E.V.A. takes care of publications and training of technicians.

SNOW AND AVALANCHE BULLETIN One of the main activities of the operators of the Avalanche Services is the collection, management and processing of the nivo-meteorological and environmental data that allow to make predictions about the stability of the snowpack.

In the Italian Alps methodologies for data collection are standardised in conformity with international ones, and are shared with others Italian authorities that deal with avalanche danger such as the Army's Service Meteomont and the Technical Committee of the Avalanche Service of CAI. The number of monitoring stations depends mainly on the climatological and orographic characteristics of the area in question: in general there is a station every 250-500 square kilometres. Regarding the altimetric dislocation, at height below 1500 m 40% of the data are taken by daily measurements, 35% by weekly measurements and 15% by automatic stations; between 1500 and 2000 m approximately 40%, 40%, 35% respectively; then above 2000 m data are predominantly collected by automatic stations (50%), the weekly surveys represent a 25% of the data and the daily ones 20%. Although these data vary greatly between different locations, they must be significant with respect to potentially hazardous slopes.

From the data collected, by hand or by fixed monitoring stations, the Snow and Avalanche bulletin is produced. The bulletin summarises the weather conditios and the state of the snow cover for the avalanche risk prediction. Each bulletin is valid within the area of jurisdiction of the Avalanche Service. This can sometimes be restrictive since the

snow and weather vary geographically and not with the political borders.

1.1.6 *Rescue authorities*

In Italy the intervention in avalanche rescue is implemented by the Corpo Nazionale del Soccorso Alpino e Speleologico (C.N.S.A.S.) and by the Soccorso Alpino della Guardia di Finanza (S.A.G.F.).

SOCCORSO ALPINO E SPELEOLOGICO (CNSAS.IT) The alpine rescue is an association of volunteers recognised and regulated by the Italian state. It was created in 1954 in Pinezolo (TN) within the Club Alpino Italiano (CAI), the oldest association of Alpine corps, under the leadership of Scipio Stenico, and it first name was Corpo di Soccorso Alpino (CSA). In 1968 the speleologic rescue joined the CSA and in 1990 the association took the name C.N.S.A.S.. In 2001 the Italian state recognised C.N.S.A.S. as a service of public utility.

The aim of C.N.S.A.S. is the rescue of people in danger, injured or fallen in alpine environments, underground and in every inaccesible place of the Country. It also contributes to prevention and cooperates with others rescue authorities.

The C.N.S.A.S. is divided in numerous regional structures whose technicians are prepared through exams and continous training. Nowadays the C.N.S.A.S. has also several canine units for avalanche rescue and for surface search. From 1955 and 2009 the C.N.S.A.S. did more than 102000 operations and helped about 118000 people.

SOCCORSO ALPINO GUARDIA DI FINANZA (GDF.GOV.IT) The mountain rescue team of the Guardia di Finanza (S.A.G.F.) was established in Italy on 30 March 1965. It was initially created to defend the border against smuggling, to patrol the border mountains and to support mountain communities.

The Alpine School of Guardia di Finanza (GdF) is much older and was established in Predazzo in 1920 for the recruitment and specialization of the entire corps in military techniques in alpine environments. With S.A.G.F., alpine rescue became more and more important.

Nowadays the corps can rely on advanced tools and equipment such as helicopters to ensure the safety, efficiency and timeliness of the rescue operation. Moreover, the expertise and the tecniques of the team are continuously updated with weeks of seasonal training organised by the Alpine School in Predazzo and Passo Rolle. There are several levels of expertise: mountain rescue technician, helicopter rescue technician, S.A.G.F. dog conductor and alpine rescue instructor.

Today the tasks of the S.A.G.F. are diversified: beside alpine rescue the corps deals with military tasks, civil protection, environmental protection, security and forensic police. There are a total of 26 bar-

raks in the whole country (Alps, Apennines and islands), with 250 military and 45 canine units for avalanche rescue, surface search and search in rubble. Since 2010, after the reform of territorial arrangements by the European Community, the S.A.G.F. has become member of the C.I.S.A.-I.K.A.R..

In the framework of the avalanche rescue operation, canine units are of particular importance. Dogs were present in the Alpine School since the beginning, in October 1965, when they started being trained in Predazzo and the S.A.G.F. barracks were 11. It is from 1991 that dogs of S.A.G.F. are trained also for surface search and the instructors started to develop novel training techniques, while in recent years the needs of civil protection in earthquakes led to the necessity of training dogs also to search rubble.

1.2 CANINE UNITS & AVALANCHE DOGS

The relationship between humans and the ancestors of the modern dog is very old. Wolf bones were found mixed with human bones already in the middle of the Pleistocene, in Zhoukoudian in China, in a site dated 300,000 years BP and in France near Nice on a site dating back to 150,000 years BP. Bones dating from 400,000 years BP were also found in Kent, England. It is likely that it was the sharing of the same hunting grounds that drove the cohabitation of the two species. Attempt at domestication could have happened already during the late Pleistocene[14–16], while the first archaeological evidence of a dog is represented by a jaw, dating back to 14,000 years ago, discovered in Oberkassel, Germany[17]. Thus, since prehistory humans have seen the dog as a potential resource.

In modern times, humans have tried to understand better the behaviour of dogs and to exploit their skills in various disciplines. Their remarkable sense of smell and predisposition to cooperate made them the ideal candidates for training. Currently the term for a dog trained to search for smells is *tracking dog*. This category includes both dogs who "work" in laboratory, also named *search dogs*, and dogs used in environments where they must follow a trail[18]. The difference between these two kind of dogs is the type of training and the working method.

In recent decades, tracking dogs have been used more frequently for the search and rescue of missing people. Previously dogs were always regarded as a military resource, and utilised for a variety of risky tasks, such as carrying messages during the two World Wars[19].

Although the use of rescue dogs is documented even before the twentieth century, it seems that the first dog involved in avalanche operations was a mongrel dog named Moritzli in 1938, whose spontaneous action gave to Swiss dog expert Ferdinand Schmutz the idea to train dogs specifically for avalanche rescue. Schmutz founded in

1940, in Switzerland, the first trained military corps in mountain rescue operations. Then, after the Second World War, the training passed into the hands of the Swiss Alpine Rescue team who still takes care of avalanche dogs (alpinerettung.ch).

The birth of the modern avalanche dog is due to American-born Bill Syrotuck who moved to Switzerland in the late 1960's and perfected the training techniques for detecting buried people in avalanches. Then as now, dogs were used because of their keen sense of smell, selectivity and accuracy that allow them to recognise the subject who emits the odour, as well as for the speed at which they can move in large difficult terrains. An interesting curiosity concerns the methods that were tried to reduce the time between the alarm and the search operation: the era of the helicopter rescue had begun.

Previously the only way to reach the injured was climbing the mountain. To drastically reduce the time reascue teams first tried to parachute the dog and its handler from the plane. But the helicopter

(a) Parachuting dog. (b) Old VS modern rescue.

Figure 2: (alpinerettung.ch).

was later to emerge as the most effective means for transporting rescuers and injured (Figure 2).

Over time the formation of avalanche dog and conductor staff has evolved greatly, especially from a technological point of view, but also in ethological studies that tried to better understand the relationship between dog and humans (see Appendix d). The term Avalanche Dog Unit (Unità Cinofila di ricerca in Valanga (U.C.V.)) now encompasses dogs and the law-enforcement staff. However, we must not think of the unit as the sum of a trainer and an animal, or of the anatomical

and physiological characteristics of dogs and men. To fully understand the Canine Unit we must think of it as a delicate collaborative relationship between members of the same herd where the handler has the hard task of maintaining the role of team leader. As a family, dog and handler live together for a lifetime and once the dog is retired it is symbolically given to the handler. Only by sharing every moment of the day is it possible to form a unity between dog and handler, and take advantage of human rationality together with a dog's instinct, hearing and sense of smell[20].

1.2.1 *Training dogs in a multidisciplinary context*

Avalanche rescue is one of the most difficult operations because there are a number of unpredictable variables during an intervention. The landscape after an avalanche is often devoid of reference points and, once reached, it is difficult to locate the detachment zone and the zone of deposit of the accident site. The weather conditions are unpredictable as is the condition of the snowpack. The burial depth can range from a few centimetres to several metres. The rescuers operates in strong emotional distress since the dogs have very little time to extract the buried alive and the canine unit may find themselves in danger too because of a second avalanche. To ensure the safety of the rescuers, sometimes it also happens that rescuers have to wait until the environmental conditions are stable before reaching the accident site. These and other unpredictable factors make it difficult to standardise training and therefore there is an objective lengthening of the time of intervention, which is critical to the success of the rescue[20, 21].

It is therefore quite clear that the success of an intervention in the event of an avalanche is linked, in addition to the intervention speed and the development of ever more sophisticated transceiver for detection, to the experience, competence, and harmony of the canine unit. To reach that harmony, some studies have investigated the effects of various training methods. First it is necessary that dogs feel work as a positive activity with their conductor. It is motivation that drives the dog to become interested in search for the buried body and therefore the motivation training begins in a dog's early years.

Since the dog considers the handler as its pack leader, the conductor must keep up a stable and linear behaviour, using an understandable and clear language in order to instill confidence and trust in the dog, with gentle but clear commands. In addition, since the dog lives with other differents canine units, it is important that every conductor has a consistent behaviour with the others. The first months of training are therefore focused on education and socialization, and the first lessons on the snow occur five to six months later.

No standardised techniques.

At this point it is important to identify methods that can teach dogs to recognise and look for a certain smell. Currently there are no standardised, tested and certified techniques, on how avalanche dogs should be trained. There are also no protocols for evaluating learning and performance during proficiency tests.

The most commonly used training techniques are based on learning by operant conditioning, and in some cases on learning by habit or by social facilitation[22–25]. In particular, instructors try to avoid approaches based on negative reinforcement or punishment because they can lead to behavioural problems such as fear and aggression. Positive reinforcement is preferred as it is considered more effective for the purpose of association of odours.

In positive reinforcement the conductor rewards the dog when it performs the requested action until it learns the right behaviour. This technique is very long and difficult, but results a collaborative and trusting dog that likes to work.

Regarding the specific training of avalanche dogs, it starts by making the dog look for its conductor buried under the snow, at a few metres distance, with various suggestions in the path. At the beginning the research has to be greatly facilitated so as to avoid any possibility of failure. Once the dog has mastered the behaviour that leads to win the game, the difficulty of the test is gradually increased by burying the conductor further away and by eliminating the suggestions. At some point, a second unknown person is buried alongside the conductor and, as the training continue, the exercise involves searching only the unknown person.

If the rules are clear and the training has been properly conducted, then the dog will be trained to search for a buried person when given the search command.

OLFACTORY TARGET During avalanche dog training the most critical part is to teach the dog to search for a specific smell. In the course of the training, an odour sample is usually presented to the dog along with other odours, and the dog is rewarded when it focuses on the *right smell*[18]. Once the dog associated the smell it starts to work on site following a simple track and the handler reward the dog when it shows search signals. It is also important to train the dog in different contexts in order to teach it to work in any environment and condition. In addition to this training it is necessary to teach the dog to communicate with the handler when the source of the smell is found. In general, the conductor teaches the dog to mark the discovery by passive signals, such as barking or staring, or by active ones such as digging[24, 26, 27].

The main problem during an avalanche is that there is specific olfactory target that can be associated with the smell of people. It is clear that the simulated discovery of a person buried under the snow

cannot be the only training system because of cost, security and availability of new volunteers. For the search of dead bodies synthetic molecules such as isobutyric acid and cadaverine are used. However, these molecules are suitable for surface research and not for avalanche research.

To overcome this problem, olfactory samples from people that are unknown to the dog are used. For example, a piece of cloth or a cotton pad is kept in the hand or under the clothes for some time, then sealed in a glass bottle and used at the time of training. Another trick is to use other objects that can be soaked with odour such as a tennis ball unther the clothes or in contact with various parts of the body. However, all these methods are potentially misleading since dogs can associate different smells beside the human one (sample contamination) and it is not yet clear whether these types of training are actually effective in increasing the ability of dogs to find people buried in avalanches[23, 27].

A significant improvement in training techniques could be achieved if canine unit trainers were provided with a kit containing molecular targets associated with human smell[28].

2

OBJECTIVES

Aim of the research is to lay the basis for the development of innovative training methods to increase the efficiency of canine units used in avalanche through the use of certified olfactory targets. To achieve the goal we work in close contact with the dog trainers and dog handlers of mountain rescue team of Soccorso Alpino della Guardia di Finanza and veterinary staff of the University of Perugia.

In this heterogeneous team, our role is to study, from the chemical-physical point of view, the feasibility of the use of specific molecules of the body odour as olfactory target for training of dogs.

1. Canine unit trainers use many differents objects and materials previously in contact with the skin as olfactory targets. Since remain unknown the major contributors to human odour, it is necessary:
   a) determine the body odour sources and among them the more appropriate for the purpose of the study;
   b) make a screening of the substances emitted by the odour source;
   c) select adequate molecular candidates for the development of olfactory target.

   To perform the analysis we rely on gas chromatography followed by electron ionization and quadrupole mass filter. The presence of a large amount of mass spectra which can be consulted make this tecniques particularly suitable for our screening purpose.

2. Once selected the potential molecular target, the diffusion processes of the analytes have to be studied in snow. Therefore we need:
   a) an optimized analytical method for the quantification of molecular targets in snow;
   b) reliable tools for investigating the diffusion processes of the molecules of interest within the snow;
   c) select the molecular targets.

   To perform diffusion studies in snow we have to develop innovative snow sampling techniques to be used in field. Moreover, as avalanche snow presents specific characteristics respect to the natural snowpack, we need to perform analysis in a simulated avalanche.

Part II

EXPERIMENTAL

3

HUMAN BODY ODOUR

ABSTRACT

In the first part of this chapter the state of the art of chemical compounds emitted by humans, identified during the last decades, is reported. The nomenclature of primary, secondary and tertiary odour and the different sources of human odour is presented. The choice of potential molecular targets according to their physical-chemical properties and specificity to human species is discussed.

Finally the analytical method for a qualitative screening of target molecules of sweat are presented and discussed. The method is based on headspace SPME on real samples followed by analysis in Gas Chromatography - Mass Spectrometry (GC-MS). Sweat samples from armpit were collected with cotton pads. Headspace extraction was performed with DVB/CAR/PDMS fiber in controlled environment. Some theoretical aspects regarding of SPME are reported in Appendix a for a better readability.

3.1 INTRODUCTION

The idea of the uniqueness of the human body odour is ancient and well presented in literature and popular culture[29, 30]. From a practical point of view tracking, trailing and the use of dogs for scent identification has been used for more than hundred years.

In the past centuries the idea of the body odour was strictly linked to religious and social belief[31, 32], but during the last thirty years there has been a lot of interest in human body odour sources[33] and a lot of work was done in order to bind molecular compouds to specific odour or aroma. The research dedicated to the analysis of the volatile organic compounds emitted by humans was done in order to achieve new insight for the diagnostic of specific deseases, for the identification of the compounds responsible for the malodour in fragrance and cosmetic research and the detection of human presence in the frame of rescue situations. A key role in this field was played by the advancement of modern analytical techniques and in particular by the development of mass spectrometry.

While there is still limited knowledge of how the body produces volatile organic compounds that characterize the human odour, it can be stated that individual body odours are determined by many factors which can be either stable in time, as the genetic factors, or may vary depending on the external environment and from the interior

of the individual physiological conditions. We can then divide the human odour into three classes[34].

PRIMARY ODOUR contains constituents which are stable in time independently from the diet or from environmental factors.

SECONDARY ODOUR contains the constituents which are present due to the diet or the effect of environmental conditions.

TERTIARY ODOUR contains the constituents that are present due to external sources such as soaps, lotions, perfumes.

Most of the literature has focused on the study of the compounds present in the underarm sweat and the soles of the feet although recently the interest in this field has led to investigate all areas of the body[35–37]. The analysis carried out in gas chromatography, coupled to IR spectroscopy or mass spectrometry, have isolated the compounds present in the extract of axillary secretions. The results of these studies[38, 39] have shown the presence of linear chains from six to ten carbon atoms, branched and unsaturated acids. Among these compounds, strong smell contributions are brought by terminally unsaturated acids, 2-methyl C_6-C_{10} acids, 4-ethyl C_5-C_{11}, along with 3-hydroxy-3-methylhexanoic acid, which is considered the major odour-causing compound. The male and female extracts are qualitatively similar but there are small differences in quantity. Short-chain fatty acid were extracted in diethyl ether through the soxhlet technique also by the feet sweat samples and in this case the isovaleric found only in those subjects who reported having a strong foot odour[40].

Other studies that have dealt with the human secretions have been conducted to identify the molecules that attract the yellow fever mosquito. In these works[41–43] a several numbers of compounds were identified as being emitted by human skin and the results confirmed the qualitative similarities and quantitative differences between subjects[44].

Studies on human smell persistence in the clothes after washing showed the absence of organic acids and the presence of other odorous compounds such as esters, ketones and aldehydes[45].

While the works mentioned above focused on the study of compounds extracted from the sweat, there are other scientific articles that are focused in the study of Volatile Organic Compounds (VOCs) present in the headspace of sweat samples[46], as the extract may not be representative of the volatile compounds emanating from the skin. These studies point out the presence of hydrocarbons, alcohols, acids, ketones, aldehydes, and have shown that the 2-nonenal is present only in individuals with more than 40 years[47]. This compound, produced by oxidative degradation of monounsaturated fatty acids such

as palmitoleic acid and vaccenic acid, is nowasdays known as the smell of old age.

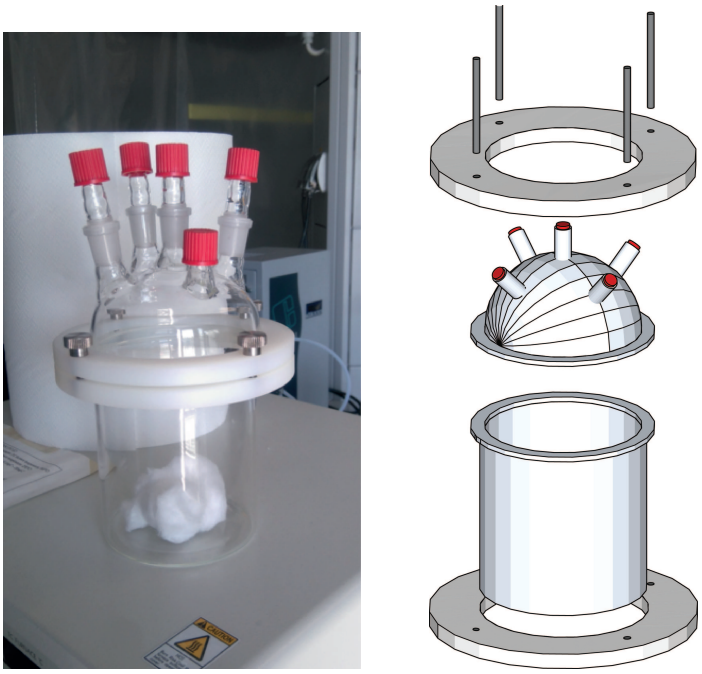
These studies confirmed that each individual may be associated with an olfactory fingerprint[34, 44, 48–51]. The abundance of common compounds vary among different people and certain subjects show specific volatile compounds, such as 6-methyl-5-heptene-2-one and short chain hydrocarbons even up to decane. It is therefore interesting to observe that is possible to associate a chromatogram to a subject by calculating the relative ratios of the common compounds. In addition, identification is more accurate if there are specific compounds that characterize a particular subject. All this evidence support the individual odour theory that is empirically demonstrated by the ability of dogs[52, 53] (but also of humans[54]) to discriminate people only according to their smell.

We try to cover as much as possible the large variety of VOCs that are cited in these articles. The Table 7 in Appendix e contains the name of the compound, the Chemical Abstract Service (CAS) number, the molecular class, weight, body source and finally the bibliographic reference of the molecule related to body odour.

3.2 CHOOSING MOLECULAR TARGETS

Between the sources of body odour, we focused in sweat since avalanche dogs are currently trained with objects that had been in contact with the skin. We focus on the molecular targets that belong to the class of the primary odours[49]. Given the success in numerous procedures[55–58] we choose the SPME to perform preconcentration of VOCs and GC-MS for conduct separation and measure. The SPME technique, invented in the 90 years from Pawliszyn, is primarily used to preconcentrate volatile analytes in the HeadSpace (HS) of a sample (HS-SPME) or analites dissolved in a liquid phase through Direct Immersion (DI)-SPME)[59]. After the extraction the fiber is directly introduced into the injector of a common gas chromatograph, without changes in the apparatus of the GC with the exception of the liner. Then the costs of the use of solvents are avoided and sample preparation is greatly simplified. A disadvantage in manual use of SPME can be the difficulty of using an internal reference standard[60], in this case the use of autosamplers simplifies and improves the accuracy of analysis[61–63].

We choose DVB/CAR/PDMS SPME fibers since they are less selective and more suitable for general screening (see also Section a.1). After choosing the target molecules, the more selective SPME is also evaluated (see Chapter 4).



(a) Picture. (b) Cad.

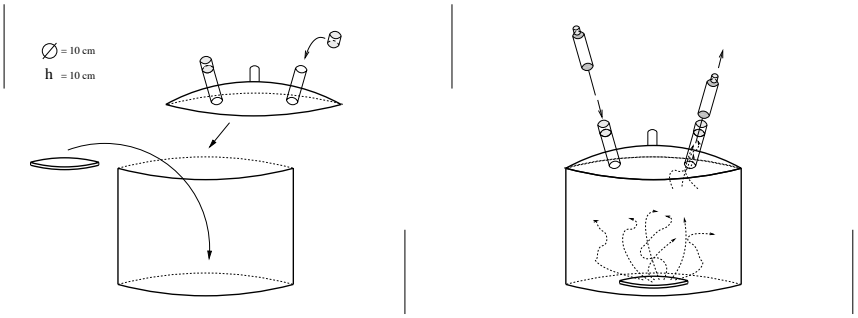
Figure 3: The reactor of our production, used to allow the extraction in the volume-controlled environment, of volatile analytes from solid samples such as T-shirts, fragments of tissue or cotton, used to sample the sweat. The base of the reactor is a beaker with frosted edges, on the cover are welded frosted borosilicate glass necks where screw caps with porosity PTFE/silicone are applied. The lid and the body of the reactor are held firmly together by two teflon rings.

VOCS EXTRACTION To perform the tests we have devised a specific borosilicate glass reactor whose main feature is to allow the simultaneous extraction of the same sample on several SPME fibers.

The idea which stands behind the manufacturing of our reactor (Figure 3) is to weld various frosted glass necks above the cover of a glass desiccator where are applied screw caps with PTFE/silicone septum. The base of the reactor is thus a beaker with frosted edges while the lid is a glass desiccator cover modified, accordingly to accommodate extraction positions. Then the lid and the body of the reactor are held firmly together by two teflon rings. We summarize here the procedure for a normal utilization of the reactor.

CLEANING The first operation is cleaning each part of the reactor before each use. For this purpose, the screws are removed from the teflon rings which hold together the body with the lid of the reactor, the cover is removed, the various glass parts are separated and decontaminated by wiping the inner surfaces with dichloromethane and hexane, the caps are finally replaced after each use.

LOADING The second operation consists in loading the sample (Figure 4a). Once dried the various parts of the reactor, the sample is positioned on the bottom of the beaker, after which the reactor is reassembled and finally closed with the teflon rings.



(a) Loading. (b) Incubation & Extraction.

Figure 4: (a)Loading of the sample inside the reactor. After decontamination and drying the sample can be loaded. Then the lid is closed with the teflon rings and the caps replaced. (b) Incubation of the sample inside the reactor and extraction via SPME fibers of the analites in the headspace. Incubation and extraction are performed for 15 hr, as incubation and extraction are performed simultaneously, in controlled environment of 25°C.

INCUBATING & EXTRACTING The third and fourth operation consists in the incubation of the sample inside the reactor and in the extraction of volatile components throught SPME fibers (Figure 4b).

3.3 MATERIAL & METHODS

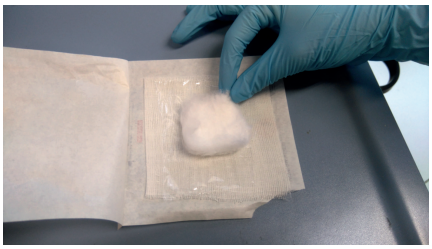
3.3.1 *Sampling*

We collected sweat samples by applying sterile cotton pads to the armpit. We used sterile hydrophilic gauze of Pharmapiù brand. Since biologically sterile does not however mean chemically cleaned, we used a cotton washing procedure in dichloromethane and hexane to exclude the presence of volatile compounds (tertiary odour) that can be misleading. The cotton pretreatment procedure consisted in washing the pads within vials of 50 ml placed in ultrasonic bath for 20 min at 50 kHz. A first washing was conducted in dichloromethane (Sigma Aldrich) followed by a wash in hexane (Sigma Aldrich) and a final wash in dichloromethane. After washing, the pads were dried under a nitrogen flow. Once dry, the swab were ready to be applied on the skin.

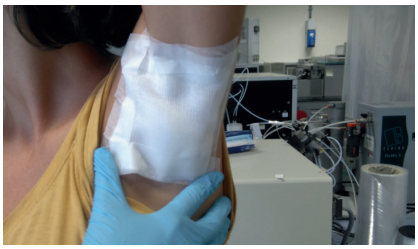
We asked the volunteers not to washing thir armpits 24 hours before the experiment, or at least to limit the use of animal soaps. In fact, in soaps produced from animal fat were found many compounds

also found in humans. Therefore we recommended the use of neutral soaps such as olive oil soap.

ARMPIT SAMPLING Six subjects were studied: three men and three women. The cotton pad was applied to the armpit (Figure 5a, Figure 5b) for 60 minutes of indoor activity. The 5x5 cm gauze was covered with a polyethylene film to minimize contamination by clothes. After sampling, the pad was placed inside the reactor, stored at room temperature for 15 hr carrying out the SPME extraction. Reactor and environmental blank samples were also taken in triplicates.



(a) Cotton Pad.



(b) Sweat sampling.

Figure 5: (a) Sterile and clean cotton swab used to collect the sweat. To prevent contamination, a polyethylene film was interposed between the cotton swab and the gauze. (b) The cotton swab was applied to the skin for sweat sampling.

The storage conditions were chosen to simulate the conditions in which the sweat is collected for canine training. It was not controlled the microbial activity on the substrate since the bacterial flora contributes to the odorous profile. The ambient temperature during sampling was 22.5°C with an average humidity of 62.1%.

3.3.2 *Extraction*

Determine the extraction time is not a simple choice since it is necessary to wait until the chemical equilibrium between the sample and the volume of the reactor is reached (see also Section a.2). However, at room temperature, the bacterial flora present in the sample acts by changing its characteristics so it is not reasonable to wait too much since the more volatile components may be degradated.

We performed degradation test in order to define the maximum extraction time avoiding the lost of the odour characteristics.

GC-MS SETTINGS We used a HRGC Agilent 6890 with an HP5-MS column, 60 m, 250 µm, 0.25 µm with helium as carrier gas at flow rate of 1.0 ml/min. The analytes were desorbed in the injector at 250°C in splitless mode. The oven temperature program begins with an isotherm at 40°C for 5 minutes, followed by a temperature ramp

of 10 C/min up to 300 C maintained for 10 minutes and followed by 2 minute post run at 305 C. Total time of analysis: 41 min. The mass spectrometer was an HP Agilent 5973 MSD with a quadrupole analyzer in EI full scan mode (range: 50 - 550). Transfer line temperature was set at 300°C, MS source at 230°C and quadrupole at 150°C.

3.4 RESULTS

The reproducibility of SPME fibers, the study of degradation of the compounds and their conservation were evaluated. Finally the compounds found in the subjects studied and their persistence in time were discussed.

3.4.1 *SPME reproducibility*

Preliminarly we tested the reproducibility of the fibers (between fibers precision). In this way it was possible to select a set of fibers with likely the same phase volume and therefore the measurements obtained could be compared. To achieve the precision between the fibers, the fibers were exposed simultaneously to the headspace of the same sample until equilibrium extraction ($t > t_{95}$) was reached (Section a.2).

The choice of using a real sample rather than a standard solution was due to the fact that using a complex matrix we take account of all possible unknowns variables that may play a role in the equilibrium extraction.

Then fibers were retracted and the signals obtained from the chromatography were compared (Figure 6).

We expressed the correlation of the instrumental response of fibers through the Pearson linear correlation index and with the coefficient of determination (Figure 7).

Pearson product-moment correlation coefficients (Equation 1) for three reproducible SPME fibers are summarized in the Figure 7.

$$\rho_{X,Y} = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sqrt{\sum (x - \bar{x})^2 (y - \bar{y})^2}} \tag{1}$$

Since the correlation index is calculated on 3681 points, in the following tests we considered comparable the experimental values obtained from this set of fibers.

We present an additional example to point out that this step is very important when quantitative analysis in SPME is needed, and that assuming the reproducibility of measurements can lead us to wrong results.

By observing the chromatograms (Figure 8, Figure 9) obtained by the desorption of three SPME fibers remained exposed simultaneously to the head space of the same sample, we can see that there are differences in the extraction of the sample components. This is an example

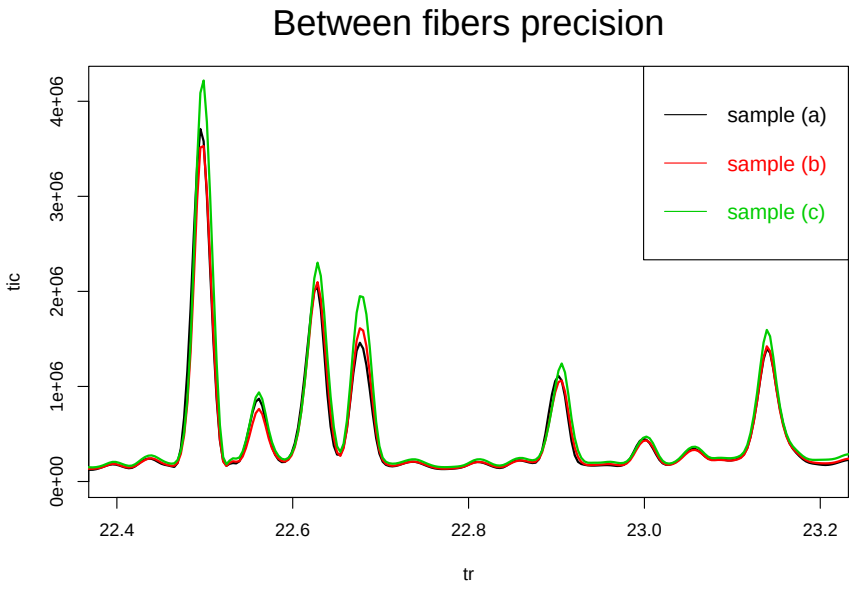


Figure 6: Comparison of instrumental response of three different (DVB/CAR/PDMS) SPME fibers of the same sample (zoom of the chromatogram from 22.4 minutes to 23.2). With this graphical test it is possible to obtain a qualitative idea of the reproducibility of the measurement between the various fibers.

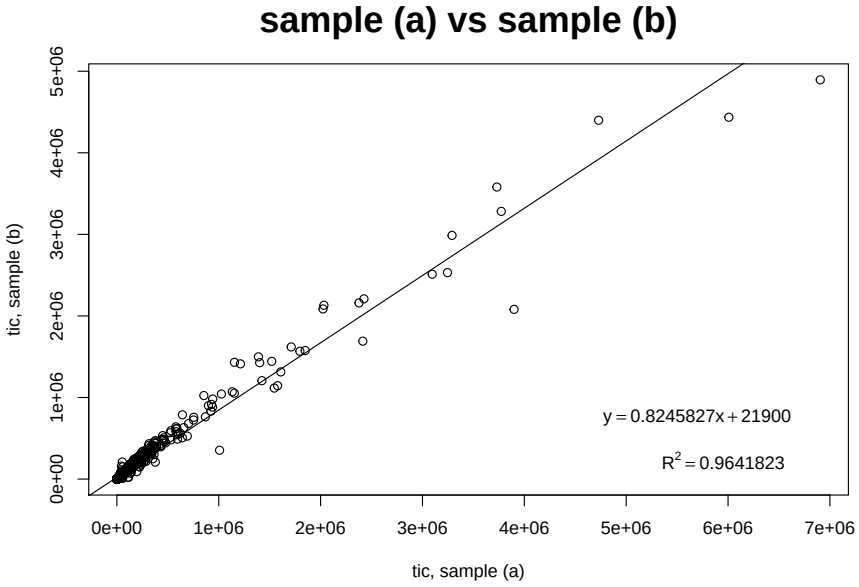


Figure 7: Linear regression model. Intercepts: 2.190e+04, slope: 8.246e-01, adjusted R-squared: 0.9642, linear correlation coefficient (Pearson): 0.9819.

Sample	$R^2_{i,j}$	$\rho_{i,j}$
sample (a) vs sample(b)	0.9642	0.9819279
sample (a) vs sample(c)	0.9717	0.9857514
sample (b) vs sample(c)	0.9771	0.9884727

in which one of the three SPME fibers gives a different instrumental response. It must be therefore taken into account the ratio between peaks area of a fiber in respect to others (if stable), or use the set of SPME fibers for qualitative measurements, or even discard the fiber if it is concluded that there are manufacturing defects.

3.4.2 *Degradation of the sample*

We conducted studies on the degradation and conservation of the sample and studied some solutions proposed in the literature[64–66].

The degradation of the samples was studied performing analysis for three consecutive days with a set of reproducible fibers. We take from the first chromatogram some reference compounds, chosen for the high value of quality index with the NIST library[67] and based on the good shape of chromatographic peak. We calculate the area of compounds and normalize for the maximum area value. The areas were then compared with the values of the next days (Figure 10).

We fitted the average of daily normalized areas with an exponential decay function:

$$y = y_0 + Ae^{-\frac{x}{t}} \tag{2}$$

where we set the offset $y_0 = 0$ and make the guess that the concentration $[c] \approx 0$ after 10 days.

From this rough model we got $A = 1.04204$, $t = 3.02016$ with $Adj.R^2 = 0.95759$. This simple model allowed us to say that $> 85\%$ of the compounds were present in the headspace of the sample for < 15 hr.

3.4.3 *Extraction time*

Equilibrium extraction in HS-SPME is reached in several hours[64, 68, 69] and in literature are reported cases in which the extraction equilibrium is reached in about 15 hours[60]. We therefore considered 15 hours a reasonable time to conduct the extraction in our analysis.

3.4.4 *Occurring compounds*

In Table 1 are shown the compounds that are the best candidates for the purpose of the research. Compounds are sorted in descending order based on the quality of NIST index. Some of these compounds, marked with ♣, were previously reported in literature. Two of these compounds were not present in the NIST library, but were recognized by the comparison[70] with the mass spectra of the standards that were kindly provided by Dr. Jean-Jacques Filippi from University of Nice.

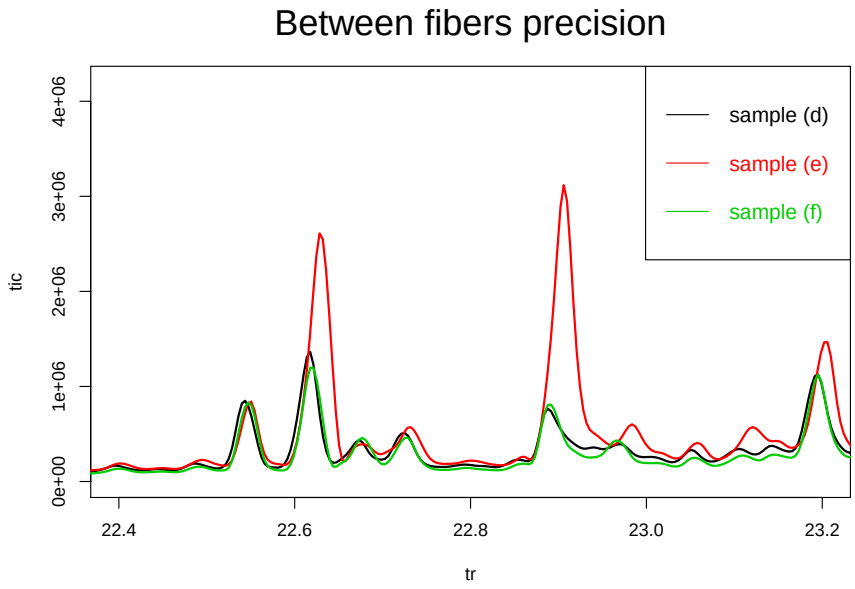


Figure 8: Comparison of instrumental response of three different (DVB/CAR/PDMS) SPME fibers of the same sample (zoom of the chromatogram from 22.4 minutes to 23.2). With this graphical test it is possible to obtain a qualitative idea of the reproducibility of the measurement between the various fibers.

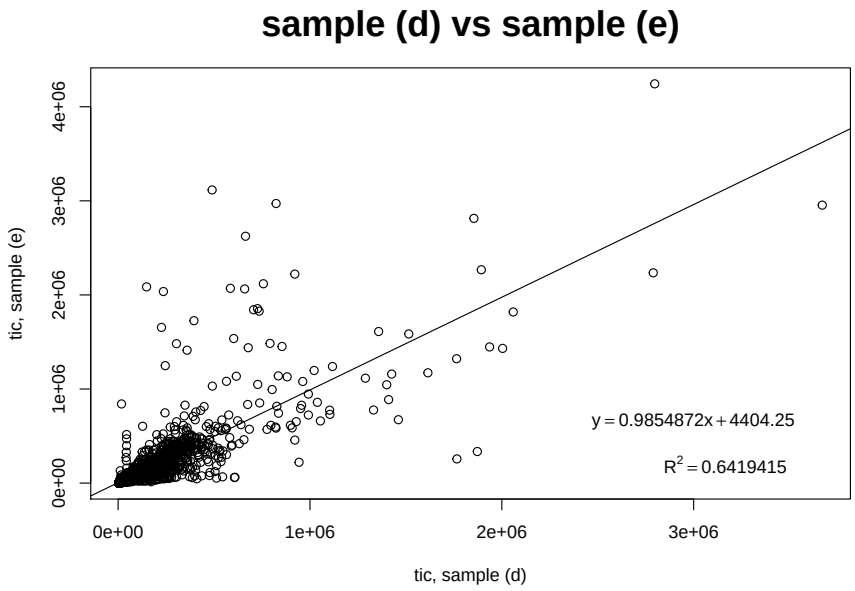


Figure 9: Linear regression model. Intercepts: 4.404e+03, slope: 9.855e-01, adjusted R-squared: 0.6418, linear correlation coefficient (Pearson): 0.8012.

Sample	$R^2_{i,j}$	$\rho_{i,j}$
sample (d) vs sample(e)	0.6418	0.8012125
sample (d) vs sample(f)	0.7244	0.8511573
sample (e) vs sample(f)	0.6459	0.8037497

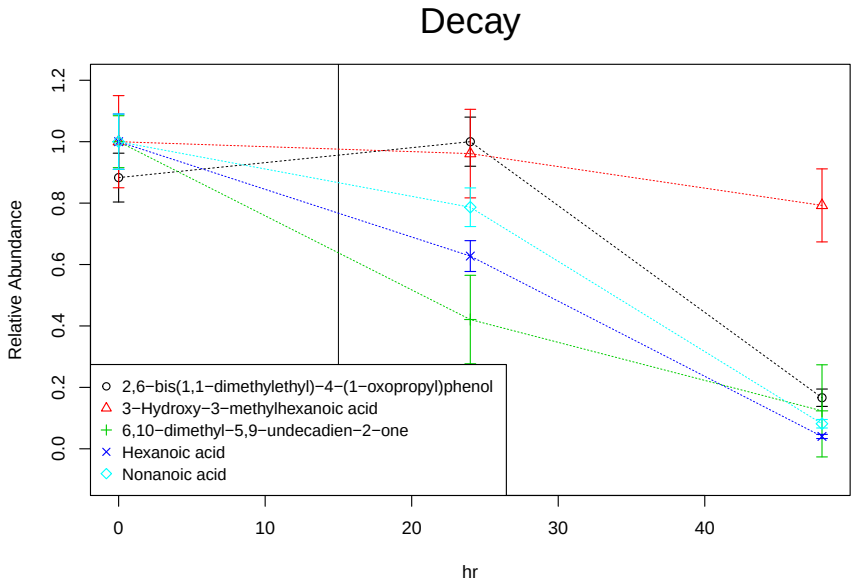


Figure 10: Degradation of sweat. Trend of the area of rapresentative ions of the molecular compounds of interest. The graph shows the stability in time of some analytes present in the sweat sample. The ions are chosen for molecules with high matching with the NIST[67] libraries and with a good peak shape. Data are normalized for the maximum area value.

		day 1	day 2	day 3
Comp	ion	$\bar{\mu} \pm \delta$	$\bar{\mu} \pm \delta$	$\bar{\mu} \pm \delta$
○	233	3216648±8%	3642716±8%	605729±5%
△	131	766344±15%	736707±15%	607454±16%
+	151	826701±7%	338506±16%	102610±15%
×	116	485650±12%	304768±17%	19533±20%
♢	115	597520±11%	469979±9%	48753±19%

ODOUR FINGERPRINT We were able to measure qualitatively and quantitatively the stability of odour fingerprint. From one hand we could observe that after a week the same subject has maintained the main characteristics of its chromatogram (Figure 11), on the other hand a quantifiable difference could be seen between chromatograms of three differents subjects analyzed (Figure 11 and Figure 12). From the comparison we can also confirm that the difference in the chromatographic fingerprints is due in minor part to the presence of heterogeneous substances, but mainly to the different relationships in the intensities of the peaks

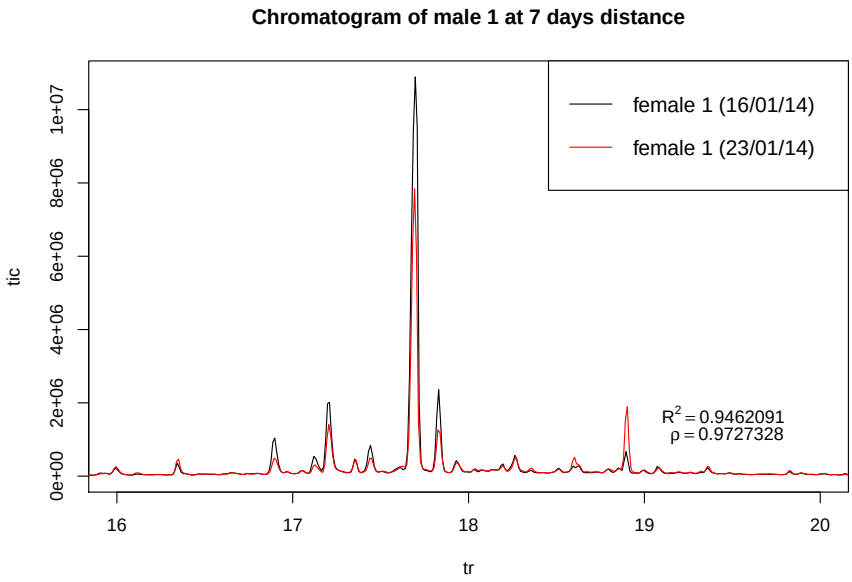


Figure 11: Comparison of chromatograms of "female 1" at one week of distance. It is qualitatively and quantitatively observable the stability of olfactory fingerprint.

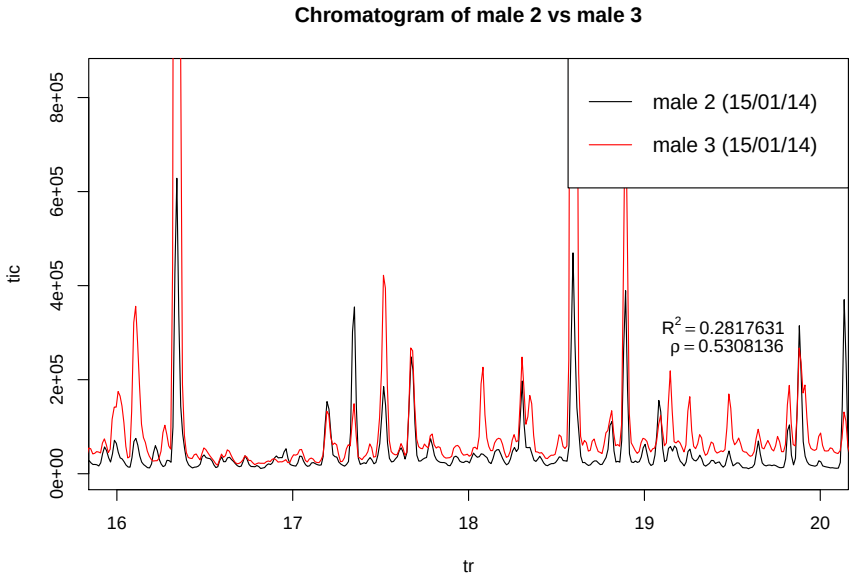


Figure 12: Comparison of chromatograms of "male 1" and "male 2". It is qualitatively and quantitatively observable the difference in odour fingerprint. It is also observable that the difference is due to the presence or absence of certain compounds (like 5,9-undecadien-2-one, 6,10-dimethyl- at tr = 18.08), but mainly to the different ratio between peaks.

3.5 DISCUSSION

In this chapter we focused in the qualitative analysis of sweat to discover potentially molecular marker of the human body odour.

From the results obtained, we can conclude that the SPME technique offers numerous advantages, compared to other techniques of extraction, for the analysis of VOCs from sweat. There are numerous methods validated in the literature, utilization is intuitive and costs are contained. Solvents are not necessary and the extraction can also be easily conducted in the environment. Moreover SPME are easily interfaced with GC-MS, the most common instrumentation in analytical laboratories. Using SPME it may be possible to encounter poorly reproducible measurements. To overcome this problem is necessary to conduct preliminary tests (especially when it is need to obtain quantitative results) to choose fibers with the same phase volume. Anyway for qualitative measure we verify that any fibers can be interchanged. Thus SPME-GC-MS is the analytical tecnique that we chose to use.

We found the presence of 3-hydroxy-3-methylhexanoic acid and (E/Z)-3-methylhex-2-enoic acid which are considered the main responsible for the sweat odour. These two acids are linked together from a chemical point of view since the latter derived from the first by the loss of a water molecule and the formation of a double bond. Moreover, 3-hydroxy-3-methylhexanoic acid is more abundant and, together with the 3-methyl-3-sulfanilhexanol[71], is the only compound of sweat that is attributable to the human race. After tests conducted in laboratory we have therefore selected the compounds that respond to our needs.

Table 1: Selection of the best candidates found in our study. Compounds are sorted based on the quality of NIST index or through comparisons[70] with the standard (st). Compound present in literature are marked with ♣.

COMPOUNDS	MATCH QUALITY
3-hydroxy-3-methylhexanoic acid♣	99 (st)
(E/Z)-3-Methylhex-2-enoic acid♣	99 (st)
Benzene, 1,3-diethenyl-	97
4-vinilbenzoic acid	97
2-furancarboxaldehyde♣	97
5-hepten-2-one,6-methyl♣	96
Hexanoic acid♣	96
Nonanal♣	96
Benzyl alcohol	95
5,9-undecadien-2-one, 6,10-dimethyl-♣	95
2,6-bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol♣	95
Phenol♣	94
Nonanoic acid♣	94
2-undecanone	92
2-furanmethanol♣	92
Hexanedioic acid dymethyl ester♣	92
2-nonanone	90
Decanoic acid	90
Diphenyl ether	90
Decanal	90
Dodecane♣	90
Benzothiazole	89
2-methoxy-4-vinylphenol	87
Butanoic acid, butyl ester	87
Octanal♣	87
5,6,7-trimethoxy-1-indanone	87
1-hexanol, 2-ethyl-	86
1-octanol	86
Hexane	86
Indole	85
3,4-diethylphenol	81
Benzoic acid, 2-hydroxy-, methyl ester	80

4

DI-SPME / GC-MS METHOD

In order to provide a reliable tool for investigating diffusion processes of the specific components of the human odor 3-hydroxy-3-methylhexanoic acid and 3-methyl-3-sulfanylhexan-1-ol through the snowpack, we developed and optimized an analytical method based on direct immersion solid phase microextraction, followed by gaschromatography – mass spectrometry[1]. Direct immersion solid phase microextraction was performed using polyacrylate fibers placed in aqueous solutions containing 3-hydroxy-3-methylhexanoic acid and 3-methyl-3-sulfanylhexan-1-ol.

4.1 INTRODUCTION

The ability of canines to track human subjects based on the latter's odor is well established. Moreover, during rescue operations carried out by avalanche dog teams, search dogs pick up traces of human scent and locate the limited area where the scent is most concentrated [73]. Some correlation (r^2=0.637) between search time and burial depth of the avalanche victims was observed [11]. This implies that volatile or semi-volatile organic compounds, specific for human odor, diffuse through the snowpack (up to several meters) until they reach the surface. Moreover, the diffusion through the snowpack also depends on the physical and chemical properties of the compound considered, such as volatility and hydrophilicity, as well as on the density of the snow and on the mean diffusion path length [74, 75].

In human secretions and excreta, a large number of volatile compounds were identified [34, 39, 49]. In human sweat, for instance, various classes of compounds, such as carboxylic acids [35, 39, 71, 76, 77], alcohols [35, 38, 39, 47, 78], aldehydes [34, 35, 47], esters [34, 35], hydrocarbons [34, 47, 79], ketones [34, 35, 47, 80], sulphur compounds [71, 78, 81–84] and terpenes [35] were detected. Although the majority of these compounds was ubiquitous, some of them, such as 3-hydroxy-3-methylhexanoic acid (HMHA) [71] and 3-methyl-3-sulfanylhexan-1-ol (3MSH) [82, 83] were identified as specific components of the human odor [71, 82, 83]. In particular, HMHA has a very typical axilla-like pungent odor and seems to be both abundant and ubiquitous in human axillary secretions [71]. Gender differences were

1 Most of this chapter is taken by the article of Dallo et al. "Direct immersion solid phase microextraction / GC-MS method for the determination of specific biomarkers of human sweat in melted snow"[72].

also observed, as females can liberate significantly higher amounts of sulphur volatiles, such as 3MSH [82].

It cannot be exclude that dog detection might be triggered by other non-specific volatiles widely excreted by humans, such as isoprene [85]. However, although the diffusion of non-polar compounds similar to isoprene from the ground to the snowpack was reported [86], the diffusion of such hydrophilic compounds toward the surface of the snowpack was not equally detailed. Hydrophilicity could affect the diffusion dynamics through the snowpack, and, in our view, HMHA and 3MSH represent suitable target molecules also for modeling the diffusion dynamics of polar and slightly polar volatiles. Indeed, since HMHA is more hydrophilic than 3MSH, different diffusion behaviors could also be expected between these two compounds.

In view of elucidating the diffusion dynamics of these human-specific markers through snow that allows, for instance, avalanche dogs to detect buried victims, a fast and reliable analytical method for the determination of these compounds in snow or in aqueous matrices (e.g., melted snow) is required.

Volatile compounds present in human sweat are generally determined by GC-MS, after extraction with organic solvents [71, 77, 84], or by headspace extraction methods [34, 49], directly applied to human secretions. Nevertheless, methods for the identification of these compounds in aqueous systems have never been reported.

Solid phase microextraction (SPME) is a solvent-free technique that simplifies the sample preparation when compared to conventional approaches: extraction and concentration are included in one single step, and the extracts do not require further clean-up procedures. Moreover, SPME generally provides low detection limits and good reproducibility both in headspace and in direct immersion (DI) mode [59, 87–89]. In DI mode, SPME was successfully tested in aqueous systems for the determination of organic compounds [90–94], proving a promising alternative to conventional extraction methods.

In this chapter, we aim to investigate the possibility of using DI-SPME/GC-MS and optimizing the operative condition for the identification of the two main specific human sweat tracers in melted snow. We focused in particular on HMHA, which is the most abundant and non-gender-specific. The analytical performances of the method were evaluated in terms of extraction efficiency, repeatability, reproducibility, linearity, limits of detection (LOD) and quantification (LOQ), accuracy. Applicability to real cases is then show in Chapter 5.

4.2 EXPERIMENTAL

4.2.1 *Chemicals and Materials*

HMHA and 3MSH were prepared by procedures adapted from published protocols [78, 84]. In short, ethyl 2-bromoacetate (130 mL; 1.99 mol) was added to a mixture of pentan-2-one (213 mL; 2.01 mol) and zinc powder (133 g; 2.03 mol) in dry THF (500 ml). The mixture was refluxed overnight and treated with a saturated solution of sodium bicarbonate. After conventional work-up, the crude oil was distilled at reduced pressure (53°C, 0.29 mbars) to yield ethyl 3-hydroxy-3-methylhexanoate (54.2 g). This ester (48.3 g) was subsequently hydrolyzed using a solution of sodium hydroxide (53 g in 460 mL ethanol/water 1/1). The reaction mixture was then washed with petroleum ether, the aqueous phase was evaporated to eliminate ethanol, acidified until pH=4, and extracted to furnish HMHA as an almost pure slightly yellow oil (42.3 g). An analytical sample was obtained by flash chromatography on silica gel using petroleum ether/ethylacetate 7/3 for elution. ^{1}H NMR ($CDCl_3$, 200 MHz): δ=0.93 ppm (t, 3H),1.20-1.65 (m, 4H), 1.27 (s, 3H), 2.53 (dd, 2H), 5.14 (br s, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ= 14.42, 17.17, 26.33, 44.13, 44.56, 71.85, 176.38 ppm. MS (EI, 70 eV): m/z (%):131 (100), 113 (14), 103 (23), 87 (52), 85 (97), 71 (38), 69 (11), 43 (67).

3MSH was prepared in four steps from pentan-2-one. At first, pentan-2-one was treated with triethylphosphonacetate in the presence of sodium hydride in dry toluene to afford ethyl 3-methylhex-2-enoate as a mixture of isomers (E/Z, ca. 75/25). This ester mixture was then refluxed for 60h with benzylthiol (1eq.) and piperidine. After evaporation of the excess of piperidine, the crude product was then reduced with $LiAlH_4$ in THF, then treated by sodium in liquid ammonia (Birch conditions) to yield crude 3MSH which was purified by column chromatography on silica gel (petroleum ether/ethylacetate 8/2) to give pure 3MSH as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 200 MHz): δ= 0.93 (t, 3H), 1.36 (s, 3H), 1.37-1.60 (m, 4H), 1.68 (br. s, 1H), 1.80-1.95 (m, 2H), 3.80-3.90 (m, 2H).^{13}C NMR ($CDCl_3$, 50 MHz): δ= 14.50, 18.01, 30.35, 46.30, 46.98, 47.63, 59.98. MS (EI, 70 eV): m/z (%): 148(5, M+), 115 (16), 114 (20), 97 (49), 87 (16), 81 (17), 71 (48), 69 (33), 55 (100), 43 (24), 41 (41).

HMHA and 3MSH were dissolved in methyl tert-butyl ether (MTBE) (Sigma Aldrich). Standard solutions were stored at -18°C until use. Sodium chloride of analytical grade was purchased from Sigma Aldrich. The ultrapure water (18.2 MΩ cm, 0.01 TOC) was produced by a Purelab Ultra system consisting of a Purelab Option R purification plant system coupled to Purelab Ultra Analytical ultra pure system (Elga, Lab Water, High Wycombe, UK). All experiments were performed at room temperature.

The SPME device for manual extraction consisted in a holder assembly and 6 replaceable fibers (Supelco) in PA (85 µm). PA fibers were selected because they proved suitable for the absorption of polar and slightly polar compounds such as carboxylic acids and thiols [95, 96]. Prior to the first use, each fiber was conditioned, as recommended by the manufacturer, by being heated in the injection port of the GC system for 1h at 270°C. After desorption of the extracts, each fiber was kept at 250 °C for 10 min in the injector, in order to ensure the complete desorption of every compound.

4.2.2 *Extraction*

SPME extraction was carried out by DI-SPME of the PA fiber into 20 mL of aqueous solution, containing 0.05-5.0 ng.mL^{-1} HMHA and 3MSH, in a 50 mL-glass vial under magnetic stirring throughout the time of the extraction. The extraction procedure was optimized evaluating the effect of the extraction time (between 0 and 180 min) , NaCl (from free-NaCl solutions to saturated) and analyte concentrations. DI-SPME extraction was carried out at room temperature.

SPME extraction in headspace (HS-SPME) mode was carried out by exposing the PA fiber to the headspace in a 50 mL glass vial over 20 mL of ultrapure water fortified with 1 and 100 ng of HMHA and 3MSH, under magnetic stirring. The PA was exposed for 120 min at 40°C.

In real snow samples (see Chapter 5), the extraction was carried out following the DI-SPME optimized procedure, where ≈20 mL of melted snow were extracted at room temperature. HMHA and 3MSH concentrations were then corrected by the effective volume subsequently measured.

The withdrawal of 50 mL of snow provides melted samples of about 10-20 mL (snow density generally ranges from 0.2 to 0.4 kg m-3 [97]). It must be noted that, considering the volume of aqueous solution, higher extraction efficiencies could be obtained in smaller vials. The choice of using 50 mL vials was done in order to minimize any possible cross-contamination or system perturbation due to phase change.

4.2.3 *Gas Chromatography – Mass Spectrometry*

HMHA and 3MSH were analyzed with an Agilent GC-MS system composed by a 6890NGC coupled to a quadrupole mass spectrometer 5973N (Agilent Technologies, Santa Clara, CA, USA). The chromatographic analysis was carried out using a capillary column DB-WAX with a 30 m length, 0.250 mm inner diameter, and 0.25 µm film thickness (Agilent Technologies, Santa Clara, CA, USA). 1 mL.min^{-1} He flow was used as a carrier. The injection temperature was investigated

in a range from 200°C to 250°C and 1 µl was injected in splitless mode. For the analysis of HMHA and 3MSH, the temperature program used was: 50°C (held for 2 to 10 min, in order to optimize the method) to 250°C at 20 °C.min^{-1} held for 3 min. The transfer-line temperature was 250°C, the Mass Selective Detector (MSD) ion source temperature was 230°C and the MSD quadrupole 150°C. Electron ionization was performed at 70 eV. Measurements in full scan mode enabled the identification of peaks. Analytes quantification was instead performed in Selected Ion Monitoring (SIM).

4.2.4 *Limit of detection, limit of quantification and linearity*

LOD and LOQ for HMHA and 3MSH were determined as the sample concentrations resulting in a peak area with a signal-to-noise ratio of at least 3:1. LOQ was the lowest concentration that could be quantified with a signal-to-noise ratio of at least 10:1 [98]. Laboratory Limits of Detection (L-LOD) and Quantification (L-LOQ) were defined as the mean concentrations of the procedural blanks plus three and ten times the standard deviations, respectively [99]. The linearity of the detector response was determined by analyzing a mixture of HMHA and 3MSH in 20 mL of ultrapure water containing analyte concentrations from 0.05 to 5 ng.mL^{-1}.

4.3 RESULTS AND DISCUSSION

4.3.1 *Optimization of thermal desorption by SPME and GC-MS analysis*

The chromatographic method was initially developed injecting 1 µL of a MTBE solution containing 5.0 µg.mL^{-1} HMHA and 3MSH separately, and recording the mass spectrum of each compound. From the mass spectrum, target ion (I^T) and two control ions (I^C) were selected for HMHA (m/z 131 (I^T); m/z 103 and 87 (I^C)) and for 3MSH (m/z 114 (I^T); m/z 97 and 71 (I^C)). Their ratios were subsequently employed for checking the purity grade of the eluted compounds. Chromatograms obtained in SIM mode (chromatographic conditions are reported in Experimental section) showed a good separation of the analytes that elute at retention time of 5.8 and 7.5 min for HMHA and 3MSH, respectively (Figure 13).

SPME desorption conditions, such as the temperature of the injector (T_{inj}) and desorption time (t_{des}), were firstly optimized by spiking 100 ng of each compound studied in 20 mL of ultrapure water. Absorption conditions (120 min of extraction at room temperature) were kept fixed during these experiments. T_{inj} of 200°C and 250°C, and t_{des} of 2, 5 and 10 min were investigated, performing injections at each T_{inj}-t_{des} combination in triplicates (n=3). Higher peak areas and more repeatable chromatograms (relative standard deviation (RSD)

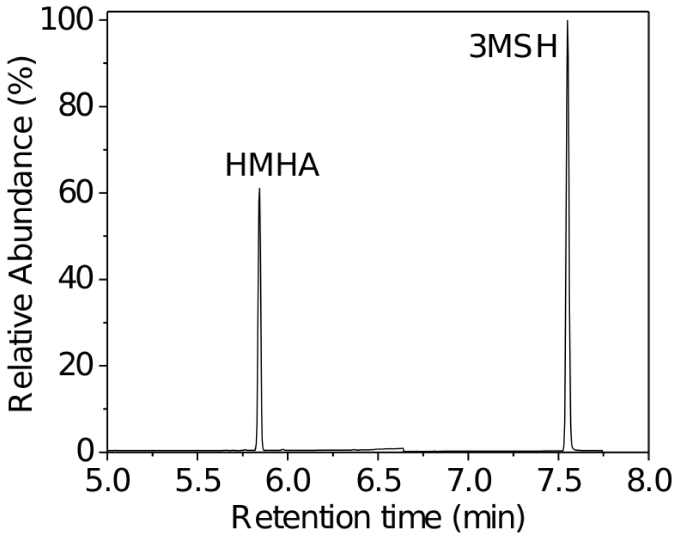


Figure 13: Chromatograms obtained in SIM mode (chromatographic conditions are reported in Section 4.2) showed a good separation of the analytes that elute at retention time of 5.8 and 7.5 min for HMHA and 3MSH, respectively.

was 6.2 %), were recorded for T_{inj} of 250°C. A lower chromatographic signal was also observed for t_{des} of 2 min when compared with 5 and 10 min. However, desorption times of 5 and 10 min provided comparable responses (see below statistical analysis), indicating that after 5 min all the analytes were completely desorbed. Thus, T_{inj} of 250 °C and t_{des} of 5 min were used as desorption conditions in the following experiments.

STATISTICAL ANALYSIS In order to determine if two series of data that follow a normal distribution are significantly different from each other, we used a statistical t-test. We decided to perform SPME extraction in 30% NaCl solution and 5 min of desorption at 250 °C after the rejection of the null hypothesis in both the two t-tests.

Since the datasets are of the same size (n=3), we performed an independent two-tailed t-test between peak area values obtained from 5 and 10 minutes of desorption at 250°C (the other data show a lower peak area average and a less reproducibility), then between peak area values from salt-saturated solutions and 30% NaCl solutions (data from salt-free solutions showed mean values much more lower).

Both t-tests were performed with 0.95 level of confidence and 2n-1 degrees of freedom. The tests were performed using the following equation:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s_{x_1 x_2}\sqrt{1/n}} \tag{3}$$

where $\bar{x}_1$ and $\bar{x}_2$ are the mean value of the first and the second set of data, respectively, n is the size of the dataset and $s_{x_1x_2} = \sqrt{s_{x_1}^2 + s_{x_2}^2}$ is the pooled standard deviation.

In table Table 2 we summarized the data collected for best temperature and desorption time, for HMHA and 3MSH, respectively. Peak area values obtained in the same solution (5 ng.ml-1 HMHA and 5 ng.ml-1 3MSH, extraction time of 120 min) after desorption time of 2, 5, 10 minutes at 200 °C and 250 °C. t value obtained for the two dis-

Table 2: Optimization of temperature and desorption time for HMHA and 3MSH.

	200°C			250°C		
Des. time	2 min	5 min	10 min	2 min	5 min	10 min
Peak Area Value HMHA	471649.75	677292.22	639268.80	630657.45	760468.46	738288.13
	601302.89	746209.68	750962.61	599357.67	816711.44	755715.53
	570258.90	635308.03	713731.34	690758.85	721732.10	830178.07
Average	547737.18	686269.98	701320.91	640257.99	766304.00	774727.24
dev.st %	12.36%	8.16%	8.11%	7.26%	6.23%	6.30%

	200°C			250°C		
Des. time	2 min	5 min	10 min	2 min	5 min	10 min
Peak Area Value 3MSH	68116.76	88904.40	93047.74	96125.66	112699.05	113882.86
	92227.67	109739.51	114593.15	81801.52	127917.29	112935.81
	85220.76	89141.16	109502.75	105596.17	107803.66	133060.64
Average	81855.06	95928.36	105714.55	94507.78	116140.00	119959.77
dev.st %	15.15%	12.47%	10.65%	12.68%	9.03%	9.47%

tributions corresponding to desorption at 5 and 10 minutes at 250 °C was t = 0.2137 < 2.132 (t95%) for HMHA, t = 0.428 < 2.132 (t95%) for 3MSH. We concluded that there is no statistical difference between these two distributions.

In Table 3 we summarized the peak area values obtained from extraction time of 120 min of three different solutions. Each solution contains 5 ng.ml-1 of HMHA and 3MSH, but differs in NaCl concentration (salt free, 30% salt and saturated). t value obtained for the two distributions corresponding to NaCl30% and saturated was t = 1.735 < 2.132 (t95%) for HMHA, t = 1.759 < 2.132 (t95%) for 3MSH. We concluded that there is no statistical difference between these two distributions.

4.3.2 *Optimization of extraction by SPME*

Absorption conditions, such as the ionic strength of the solution and absorption time (t_{abs}), were also investigated. Indeed, it was recognized that the ionic strength of the aqueous solution generally affects the yield of extraction [100, 101]. The increase in ionic strength of the solution was achieved by adding sodium chloride. The ionic strength conditions was optimized in 20 mL ultrapure water solutions spiked with different amounts of HMHA and 3MSH (5.0, 2.5, 1.0, 0.25, 0.05 ng.mL^{-1}), where each solution contained either 30% NaCl ($NaCl_{30\%}$)

Table 3: Optimization of salt concentration for HMHA and 3MSH.

	$NaCl_{free}$	$NaCl_{30\%}$	$NaCl_{sat}$
Peak Area Value HMHA	39395.27	760468.46	773782.21
	48437.85	816711.44	884502.11
	44522.99	721732.10	873325.14
Average	44118.70	766304.00	843869.82
dev.st %	10.28%	6.23%	7.22%
	$NaCl_{free}$	$NaCl_{30\%}$	$NaCl_{sat}$
Peak Area Value 3MSH	63165.42	112699.05	132470.87
	52378.76	127917.29	120345.81
	49993.18	107803.65	145463.94
Average	55179.12	116140.00	132760.21
dev.st %	12.72%	9.03%	9.46%

or was saturated ($NaCl_{sat}$). For comparison, the same HMHA and 3MSH solutions were also prepared in salt-free solutions ($NaCl_{free}$). In this set of experiments, tabs was kept at a constant value of 120 min. The peak area values obtained at different HMHA and 3MSH vs the concentration levels and NaCl contents are reported in Figure 14a and Figure 14b, respectively. As expected, the peak area of both HMHA and 3MSH considerably increases in presence of NaCl. However, in NaCl-saturated solutions, a higher increase of the extraction yield was observed for HMHA (≈18-22 times higher) rather than 3MSH (≈1.9-2.4 times higher), respectively, suggesting that the partition coefficients of these two compounds changes depending on the ionic strength of the solution. This behavior is consistent with the higher hydrophilicity of HMHA. The use of NaCl-saturated solutions does not significantly improve the extraction yield, when compared with NaCl30% (see statistical analysis in Supporting Material). Furthermore high NaCl concentration levels could shorten the lifetime of the fibers [102]. Thus, in this study, we considered that a NaCl concentration of 30% provides a useful balance between the increase of the extraction yield and the decrease in lifetime of the SPME fibers, and we therefore employed it in the following experiments.

The effect of t_{abs} was evaluated by keeping constant the ionic strength of the solution ($NaCl_{30\%}$), HMHA and 3MSH concentrations (100 ng spiked in 20 mL of water), while t_{abs} was varied between 10 to 180 min. As elsewhere demonstrated [103], the amount of analyte absorbed at the equilibrium (N^0) resulted to be a function of the t_{abs}, according to the following equation:

$$\frac{N}{N^0} = 1 - e^{-at_{abs}} \tag{4}$$

where N is the amounts of analyte absorbed at a time t and a is a parameter that determines the absorption equilibrium rate at the fiber surface.

In Figure 15 the amount of absorbed analytes (N/N^0) versus t_{abs} is reported and fitted with Equation 4 for both HMHA and 3MSH. The non-linear fit curve for these values provided adjusted-r^2 of 0.935 and 0.997, for HMHA and 3MSH, respectively. The values obtained for the parameter a (0.028 for HMHA and 0.006 for 3MSH) indicate that the absorption equilibrium was reached faster for HMHA than for 3MSH. From the fitted equations, the times required for absorbing the 95% respect to the equilibrium conditions were 107 and 499 min for HMHA and 3MSH, respectively. Although the absorption equilibrium of 3MSH was quite low, t_{abs} of 120 min was considered suitable for determining these two compounds, shortening the analysis time and obtaining nearly the total amount of extractable analyte for HMHA (96%) and 51% of 3MSH. The optimized absorption and desorption conditions of the method are: t_{abs}=120 min; NaCl concentration=30%, t_{des}=5 min and T_{inj}=250°C. These conditions were used in the experiments reported in this paper.

Optimized absorptions and desorption condition: t_{abs}=120 min; NaCl concentration=30%, t_{des}=5 min and T_{inj}=250°C

The stability of the analytes was also investigated, considering 20 mL ultrapure water fortified with HMHA and 3MSH (5 ng mL-1) kept at room temperature and analyzed daily for 3 days. In these conditions, whilst the HMHA signal did not vary significantly (RSD calculated between 3 daily measurements was 5%), the peak area, corresponding to 3MSH, progressively decreased as shown in Figure 16. After 24 hours, the signal was 80% of the initial value. The possibility of preserving 3MSH in aqueous environments was investigated by freezing 20 mL of spiked samples, stored at -18°C and analyzed after 30 days. As reported in Figure 3, the analyses performed immediately after thawing the spiked water samples showed that the chromatographic signal of HMHA did not significantly vary (within 6%). Moreover, 3MSH signal appeared only slightly reduced (15%) with respect to the signal initially obtained. However, 1 day after thawing, the same decreasing trend was observed. The behavior of 3MSH could be due to degradation phenomena in aqueous environment, as suggested by the oxidation of thiol compounds in aqueous solutions containing molecular oxygen [104, 105]. These oxidation reactions are likely slowed down when freezing the samples. In light of these results, we concluded that storing the aqueous samples at -18°C, until SPME analysis, ensures the reliability of the measurements.

The extraction efficiency of the method was evaluated considering the ratio between the peak area of the analytes extracted using DI-SPME in 20 mL of ultrapure water and the corresponding values obtained when injecting MTBE solutions in which the same amounts of HMHA and 3MSH were present. The major assumption is that the chromatographic signal is not affected by the presence of the

solvent during the GC-MS analysis performed in splitless mode. In our view, this approach leads to a direct comparison between the amount of the analyte extracted and the amount of analyte initially present in the aqueous solutions. A set of experiments was carried out at two different HMHA and 3MSH concentration levels (0.25 and 5.0 ng.mL^{-1}). As reported in Table 4, the extraction efficiency ranged between 10-12% and 2-3% for HMHA and 3MSH, respectively, depending on the concentration level. The extraction efficiency values obtained are comparable to similar methods that use DI-SPME extraction for the determination of other compounds, such as polycyclic aromatic hydrocarbons [106], phthalate esters citehou2015polyethylene, farajzadeh2015microextraction and pesticides [107]. It must be noted that, in several studies [106–108], the extraction efficiency was considerably improved when using HS-SPME extraction instead of DI-SPME, especially when analyzing non-polar compounds. When extracting the headspace of a 50 mL vial in similar experimental conditions than those used in DI-SPME (T was 40°C instead of room temperature), extraction efficiency values of 2% and 7% were obtained for HMHA and 3MSH, respectively, at 5.0 ng.mL$_{-1}$. The increase of the extraction efficiency for 3MSH in HS-SPME, differently from HMHA, is consistent with the hydrophobicity of 3MSH, that preferentially distributes in the gas phase rather than aqueous. However, although the extraction efficiency of 3MSH increased, HS-SPME procedures proved scarcely reproducible for this latter compound (RSD>80%, n=6). In this paper, considering the potential applications of the method, we opt for higher precision more than higher extraction efficiency. Thus, in light of these considerations, DI-SPME results more suitable than HS-SPME for HMHA and 3MSH extraction in an aqueous matrix.

4.3.3 *Analytical performances of DI-SPME/GC-MS method*

The within- and between-day precision was determined as RSD at high (5.0 ng.mL^{-1}) and low (0.25 ng.mL^{-1}) concentration levels of HMHA and 3MSH in 20 mL ultrapure water, repeating the DI-SPME/GC-MS procedure in 6 replicates for each concentration level and using the same SPME fiber. As shown in Table 4, the within-day precision for HMHA and 3MSH at higher concentration levels did not differ significantly (8% and 10%, respectively), indicating an acceptable repeatability of the method. At lower concentration levels, the within-day precision slightly increased (see Table 4). The between-day precision showed similar results when using fresh spiked solutions (Table 4).

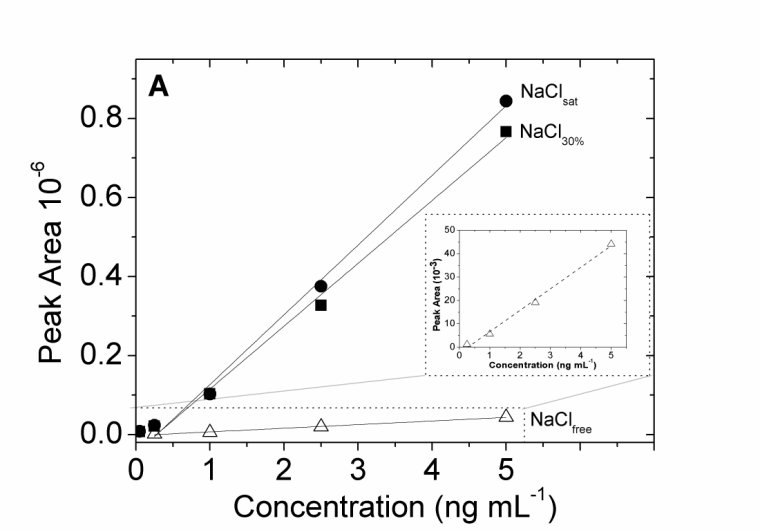
The reproducibility of the method was also investigated considering the variability between a batch of 6 different fibers at high (5.0 ng.mL^{-1}) and low (0.25 ng.mL^{-1}) HMHA and 3MSH concentration

levels (see Table 4). The concentration levels were chosen considering the LOQ values (see below). In particular, the lower concentration was set close to LOQ values, while the higher concentration was set at about one order of magnitude above the LOQ. At high concentrations, the variability between the fibers was 10% for HMHA and 12% for 3MSH, a result comparable to within-day and between-day precision values. At low concentration levels, the variability was 15% for HMHA and 18% for 3MSH. However, it must be mentioned that the RSD values obtained at low concentration levels referred to 5 SPME fibers. Indeed, peak areas obtained with one SPME fiber were outliers, based on the Dixon's Q test [109] and were therefore rejected.

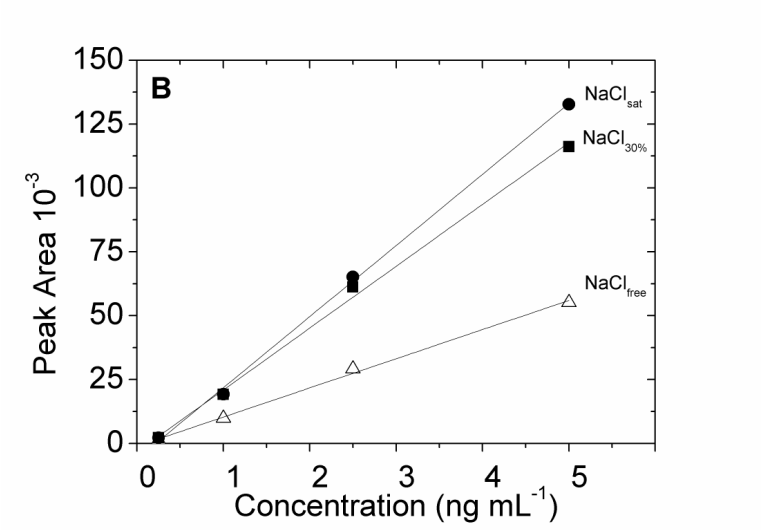
Linearity, LOD and LOQ were determined as described in the Experimental Section. Calibration curves were obtained in a concentration range 0.05-5.0 $ng.mL^{-1}$ for HMHA and 0.25-5.0 $ng.mL^{-1}$ for 3MSH. The concentrations compared to the chromatographic signal are linear when using up to 5 $ng.mL^{-1}$ for HMHA and 3MSH. LOD (LOQ) were: 0.01 $ng.mL^{-1}$ (0.04 $ng.mL^{-1}$) and 0.06 $ng.mL^{-1}$ (0.20 $ng.mL^{-1}$) for HMHA and 3MSH, respectively.

The contribution of the laboratory environment to the procedural blanks was evaluated by extracting in replicates (n=6) 20 mL ultrapure water solutions containing 30% NaCl. In procedural blanks, for retention times corresponding to HMHA and 3MSH, the concentration of the analytes was less than three times the S/N ratio in all the replicates.

Since real samples are melted snow that potentially differs from pure water, we considered the possibility of matrix effect during analysis. An accepted method for evaluating matrix effect uses isotopically labeled internal standard calibration method [110–112]. Unfortunately, isotopically labeled HMHA and/or 3MSH are not available. Matrix effect was then evaluated considering the matrix (real melted snow, 20 mL) fortified with HMHA and 3MSH (0.25 $ng.mL^{-1}$ and 5.0 $ng.mL^{-1}$). In triplicate for each concentration level. Melted snow was previously analyzed (see field blanks in section 3.3). As reported in Table 4, the recovery ranged from 89% to 95% for HMHA and from 84% to 101% for 3MSH, depending on the concentration level considered. These results indicate that, although for low concentrations (0.25 $ng.mL^{-1}$) the recovery was slightly lower than 90%, for higher concentrations (5.0 $ng.mL^{-1}$) the matrix does not really affect the recovery accuracy of the measurement. It must be noted, however, that snow is a matrix that could be considerably different from one sampling site to another. Thus, in our view, the evaluation of the matrix contribution should be performed in every specific sampling site.



(a) HMHA



(b) 3MSH

Figure 14: Peak area vs concentration of HMHA (a) and 3MSH (b) at different NaCl concentration: saturated NaCl (•), 30% NaCl (■) and salt-free solution (△).

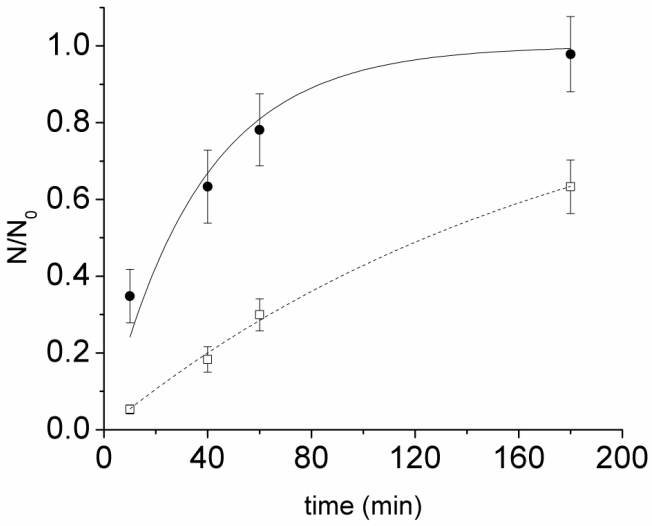


Figure 15: N/N^0 vs time at the SPME fiber of HMHA (●) and 3MSH (□) in solution containing 5 $ng.mL^{-1}$ of analytes and 30% NaCl. Fitted Equation 4 for HMHA (—) and 3MSH (---)

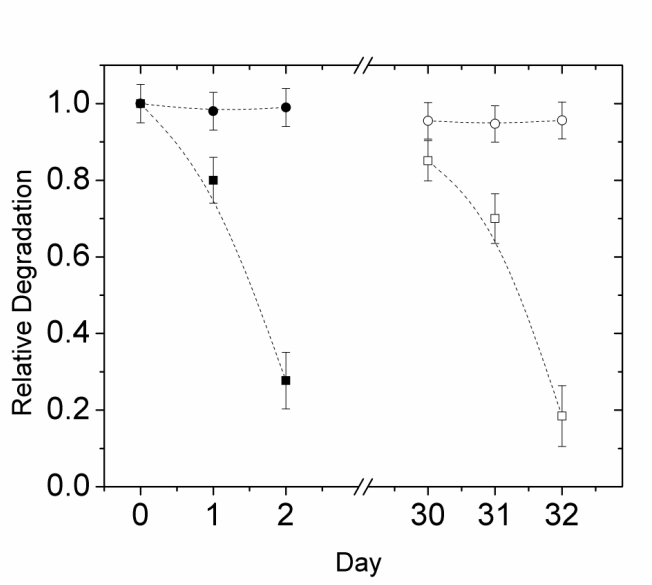


Figure 16: HMHA (●) and 3MSH (■) stability in water. HMHA (○) and 3MSH (□) stability in water after 30 days storage at -18°C.

Table 4: Analytical performances of the method. (a) and (b) referred to HMHA and 3MSH, respectively.

Compound	Extraction Efficiency %		Within-day precision RDS (n=6)		Between-day precision RDS (n=6)		Between-fibers precision RDS (n=6)		Accuracy %		LOD ng/ml	LOQ ng/ml	Linearity
Concentration Range ($ng.mL^{-1}$)	0.25	5	0.25	5	0.25	5	0.25	5	0.25	5			0.05 - 5[(a)] 0.25 - 5[(b)]
HMHA	10.2	11.9	12.4	7.8	11.8	8.5	15.2	10.4	89.0	95.1	0.01	0.04	0.979
3MSH	2.3	3.2	14.1	10.5	16.3	10.8	17.7	11.6	84.4	100.8	0.06	0.20	0.973

4.4 CONCLUDING REMARKS

In this chapter, a novel DI-SPME/GC-MS method for extracting HMHA and 3MSH from aqueous systems was developed and optimized in terms of absorption and desorption conditions. The method does not need extensive sample treatment (extraction and clean-up procedures) and provided within- and between-day precision in the range of 8-16%. Although the extraction efficiency values ranged between 2-12%, depending on the analyte considered, the method is suitable for analyzing aqueous solution containing at least 0.04 ng mL^{-1} and 0.20 ng mL^{-1} of HMHA and 3MSH, respectively.

We also demonstrated the applicability of the method to melted snow samples fortified with HMHA and 3MSH. This method opens the possibility of studying and modeling the diffusion mechanism of these compounds through the snowpack toward the atmosphere.

5

VOCS DIFFUSION IN SNOW

Before considering whether our markers are suitable molecular targets for the training of dogs, it is necessary to study their diffusion within the snowpack to be certain that these odours can be smelled within a reasonable time after burial.

The huge number of possible combinations of the variables that characterize the snowpack and their constant variability, make this study a highly complex one. We designed and developed an innovative procedure which allowed us to simulate the conditions in which human odours are diffused through avalanche snow. The diffusion environment for HMHA and 3MSH was recreated, as if these molecules were issued by clothes in contact with a warm body at 50 cm depth. To highlight the diffusion of targets in the snow, samples at differents depths on the vertical of the targets were analyzed with the method described in Chapter 4.

A side experiment to simulate the breathing of a buried victim with a system capable to pump air into the snowpack (see Appendix c) was also carried out and tested directly with the Canine Units of the S.A.G.F..

The results of these experiments are encouraging and allowed us to obtain consistent experimental data that are necessary for the description of a complete model of diffusion of human VOCs in particolar snow conditions.

5.1 INTRODUCTION

We present here a very concise summary of the main characteristics and properties of the snowpack as an Introduction to the discussion of the results.

5.1.1 *Snowpack properties*

The snowpack is composed of ice, air, water and particles. It can be seen as a porous surface that is relatively homogeneous when compared to the ground, but a more detailed analysis of seasonal snow shows that the snow is actually a superposition of layers containing different snow crystals, which are more or less homogeneous within their boundaries[113]. The heterogeneity of snow is due to the accumulation of new snow above old layers and later by the metamorphic processes of the snowpack due to thermal gradient, wind speed, relative humidity, and solar radiation[114, 115].

DESCRIPTION	CODE[116, 117]	SYMBOL
Precipitation particles	PP & 1	+
Machine Made snow	MM	◎
Decomposing and Fragmented PP	DF & 2	/
Rounded Grains (monocrystals)	RG & 3	•
Faceted Crystals	FC & 4	□
Depth Hoar	DH & 5	⋀
Melt Forms	MF & 6	○
Surface Hoar	SH & 7	⋁
Ice Formation	IF & 8	■
Surface crusts	9	∀

Table 5: Main morphological grain size classes[116, 117].

Different types of snow grains can be identified through direct observation by an expert nivologist. Snow grains are classified according to the Size of grain (E) and the Form of grain (F). There are nine major classes for the shape of grains (see Table 5), split in turn into numerous subcategories[116, 117]. As grains are often irregular, their size is definited by measuring the average length of the larger dimension of some characteristic grains of the layer[113]. The size of the crystals go from *very fine* (when < 0.2 mm) to *extreme* (5 mm).

Both E and F are important parameters and contribute to many properties of the snowpack, such as density and the Specific Surface Area (SSA). Density refers to the mass of snow per unit of volume and is easily measured in the field. The SSA is more complex to measure but it is a recently studied fundamental property that determines all the adsorption interactions between gasses and snow crystals[118]. The measurement of the SSA involves the adsorption of methane according to the isotherm of Brunauer-Emmett-Telle. Generally SSA falls between 100 and 1500 cm^2g^{-1}, depending on the type and aging of the snow. It is also possible to estimate the SSA from E, F, and the density of the snow[119, 120]. Finally, the Total Surface Area (TSA) of the snowpack can be estimated as a product of SSA, density and total snow depth[120].

The snow is a highly transient medium whose physical properties change rapidly with time, even within a few hours. In addition, the temperature fluctuation of the snowpack changes in 24 hr and with this E, F, density, SSA and consequently also the adsorption equilibria at the grain boundaries. It was observed that the SSA decreases as the snow ages and that the speed of decrease accelerates in periods with high temperature and high wind speeds. The mechanism by which

SSA decreases is the rounding of the grains, the sublimation of the grains and the formations of a Depth Hoar[121].

5.1.2 *Thermal gradient*

The temperature of the snowpack, and especially of the superficial snow, is not constant.

The snow cover is included between the ground and atmosphere. The geothermal and solar radiation heat that accumulates in the soil, expecially during summer, warms up the basal layers of the snowpack up to 0° C. On the surface the snowpack is influenced by the air temperature, which is cold during the winter season, and is also subjected to temperature variations due to the day and night cycle. The inner layers of the snowpack remain rather isolated. Therefore the surface of the snow is on average colder than the most internal layers, and the base of the snowpack is at 0 ° or at slightly lower temperatures.

The effect of such differences in temperature is the formation of a thermal gradient, a vector quantity measured in °C/m, which in the snowpack is conventionally directed towards the highest temperature, (then generally downwards). The lack of thermal gradient is a particular case of wet snow at a uniform temperature of 0°C.

The thermal gradient and pressure contribute to the metamorphism of snow, a fundamental process that changes the physical properties of the snowpack according with the changes in F and E of its grains.

5.1.3 *Metamorphism of dry snow*

Once deposited, snow crystals immediately begin to transform. At first, the snowflake loses its ramifications and adopts a more rouded shape, a preferable form in thermodynamic terms because of the low surface/volume ratio.

This first phase, called destructive metamorphism since there is a reduction of the average size of the particles and the disappearance of their ramifications, is followed by a phase of constructive metamorphism in which the size of the particles tends to increase. This phenomenon is caused by the sublimation of water vapor from the grains of the lower layers of the snowpack (warmer) and the condensation of water molecules in the grains of the upper layers (colder). Since the water vapor in motion due to the thermal gradient tends to condense into larger particles where the vapor pressure is low, there is the formation of layers with larger particles that grow at the expense of smaller particles in the lower layers.

The process can be summarized as follows. In a typical situation the snow in contact with the ground is at zero Celsius degrees. The temperature drops down at the surface since the surface is exposed

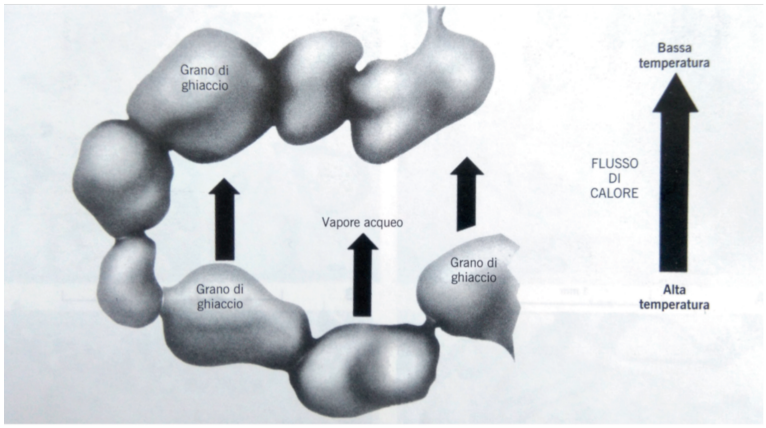


Figure 17: The heat flux, moving along with the water vapour flux, from the bottom to the top of the snowpack. The water vapour condense on the bottom of the snow grains of the upper layer while sublimate from the top of the grains of the bottom layers. This process is also known as *hand to hand*. Image from McClung and Schaerer[1].

to the cold air of winter. As hot air can hold more water vapor than cold air, there is a higher concentration of water vapor in the pores present at the bottom of the snowpack. The water vapor then rises towards the surface through the pores, sublimating from a grain and condensing on a higher one. So the diffusion of water vapor takes place through a hand-to-hand process in dry snow. The speed of diffusion, related to the thermal gradient, determines the shape of the crystals that develop within the snowpack (see Figure 17).

5.2 EXPERIMENTAL

5.2.1 *Diffusion of standards*

To study the diffusion in the snow, it is necessary to have a reproducible sampling system and a standardized sampling procedure. To this aim we developed a snow corer (Appendix b) whose innovative characteristic is the possibility to make available the inner snow sampled.

To simulate the conditions of a snow avalanche, and to break the layers of the snowpack, we prepared an artificial avalanche deposit site where the analysis could be performed. Snow was accumulated by using the snow cat of the Guardia di Finanza under the supervision of an expert nivologists from ARPAV.

SNOW SAMPLING The coring procedure consists in few operations with the corer. All the operations have to be done with great care. First we place the corer upright on the snow and start to penetrate

the snow, holding the corer from the handles, until 0.5 m of depth is reached (Figure 18a). Graduated signs on the body of the corer indicate the depth reached. Next the core is *slowly* pulled out of the snowpack, leaving a hole in the snowpack, because a fast extraction could modify the conditions of the snow inside the barrel. To prevent the leak of snow from the bottom, a few threads in the inner part of the corer head there are some threads that perform a slight friction on the snow. In very few cases, as in the presence of fresh snow on the bottom, the coesion of grains is not sufficient and the snow may be lost when pulling off the corer.

Once extracted, the corer is laid down on a flat surface and we start to disassemble the head (Figure 18b). Once the head is separated from the body and emptied of snow, we can put the heat source inside it. This is done to simulate the human body temperature (Figure 18c). The heat source consists of a 150 ml thermos filled with water at 37° celsius and with an internal thermometer (Figure 18d). We used the HOBO 8K Pendant® Temperature/Alarm (Waterproof) Data Logger to monitor the temperature of the heat source. In order not to spill water the termos is wrapped with polyethylene film. Above the polyethylene we placed the odour source which consists of a disk of cotton soaked with HMHA and 3MSH (Figure 18e).

The corer is then reassembled by placing the body of the corer containing the snow on the head containing the heat and odour source (Figure 18f). To prevent the escape of volatile compounds, the whole corer except for the handle is wrapped with polyethylene film. Then the corer is placed into the hole and the outer parts of the corer are covered with snow, taken in the vicinity of the sampling site, to protect the corer from solar beams (Figure 18g). The corer is held inside the snowpack for the time of the diffusion test, then is extracted and completely disassembled (head and handles) to allow the opening of the barrel containing the snow (Figure 18h).

5.2.2 *Melted Snow Samples*

Snow samples were collected at Passo Rolle (Trentino-Alto Adige, Italy, 46°17′48.6"N 11°47′15.6"E) on 12 March 2016, using the snow corer. A snow core (0.5 m length) was drilled in correspondence with an olfactory target, consisting of a cotton pad soaked with 500 ng HMHA and 3MSH respectively, previously placed at the bottom of the core together with a thermal source consisting of a bottle of warm water (37°C) in order to simulate the thermal input of avalanche victims. This allowed the human sweat biomarkers to diffuse through the snow. The snow core was sub-sampled by collecting ten sections (5 cm length) in 50 ml vials. The samples were stored below 0°C during the field campaign, then transported inside a cooler and stored in laboratory at -18°C until analysis. Four tests were performed leav-

(a) Sampling of superficial snow.

(b) Removing the drill head.

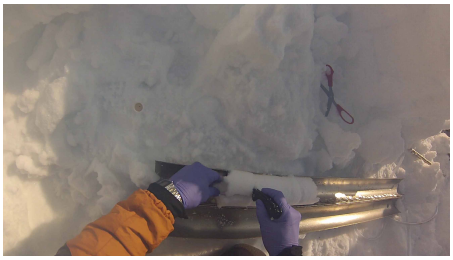
(c) Head cleaned from snow.

(d) Thermometer inside the water.

(e) Soak the cotton with standards.

(f) Rebuild the corer.

(g) Wait the diffusion time.



(h) Access the snow.

Figure 18: Summary of the main operation for the snow sampling with the corer.

	MORNING	NOON	AFTERNOON
Temperature °C	$-4.4 \pm 12.0\%$	$-3.6 \pm 9.6\%$	$-2.3 \pm 28.2\%$
Density kg/m^3		$366 \pm 4.8\%$	
Grains		RG/3	

Table 6: Summary of the properties of the snow used to perform analysis. The percent standard deviation in the afternoon is large due to the temperature of the snow surface. If the value of superficial temperature is not used, average and percent standard deviation become more reasonable: $-2.6 \pm 8.3\%$.

ing the corer with the odour source in the snow for 20, 40, 60 and 80 minutes.

5.3 RESULTS

5.3.1 *Snowpack properties*

Since the accumulation of snow is uniformed by the snowcat, the layers of the snowpack and the thermal gradient are lost. In our view this is the most appropriate way to avoid the spatial variability of the snowpack and to limit the number of variables involved in the experiments to density and temperature.

We checked the density, the snow temperature and the type of grains present in the accumulation to confirm the uniformity of the surface of the snowpack (50 cm) in three representative moments of the day: in the morning at 9, at 12:30 and in the afternoon at 16. The total amount of accumulated snow height is 1.80 m. The snow was checked from the surface down to 50 cm depth every 10 cm (n = 6).

In Table 6 we summarized the measurements. We observed that in the morning the snow is colder due to night cooling; at noon the snow is slightly warmer; however the maximum temperature of the snowpack is reached in the afternoon due to the accumulation of heat during the day. The percent standard deviation in the afternoon is large due to the temperature value of the surface (-1.2 °C). If the temperature in the immediate vicinity of the surface is not considered the mean value becomes -2.6 and the percent standard deviation is 8.3% . These data are comparable to other values obtained.

The density of the snow accumulated on the surface remains the same since there is no strong thermal gradient in the snowpack capable of generating a quantifiable constructive metamorphism. The density of the snow is higher than the virgin snow since it is compacted by the snowcat.

The snowpack was characterized by RG or 3 grains, depending on the symbology used. This was in accordance with the nivo-metereological

data as the snow used to produce the simulated avalanche presented rounded grains formed after the distructive metamorphism of the snowfall of 2, 5 and 7 March.

5.3.2 *Temperature of the heat source*

A typical trend of the temperature inside the heat source is shown in Figure 19. The temperature is measured every 10 seconds. We can observe that in about two minutes the thermometer reaches the water temperature, then the heat source is cooled exponentially. Since the measurement is not instantaneous we should take into account the delay with which the probe responds to the change of the water temperature.

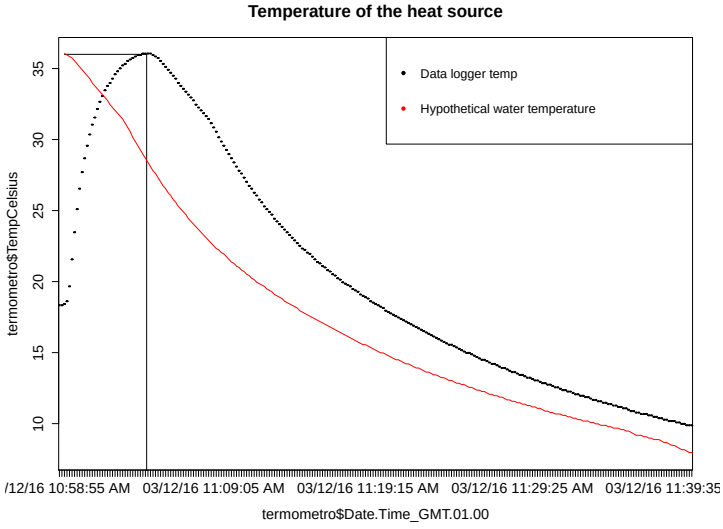


Figure 19: Typical trend of the temperature inside the heat source. The red line represents the real data shifted to the left so as to indicate the exponential decay of the water inside the heat source.

5.3.3 *Melted Snow Analysis*

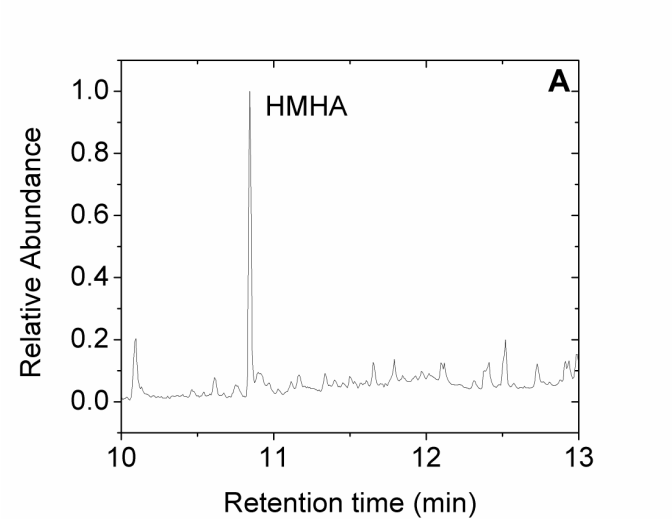
Diffusion studies of hydrophilic semi-volatile compounds through the snowpack were performed. Field blanks (n=4), consisting in surface snow collected nearby the coring spot, were firstly analyzed. In every sample, the chromatographic signals in correspondence with the retention times of HMHA and 3MSH were below the LOD.

Typical SIM chromatograms recorded in real samples of melted snow subsampled at 5 cm and 15 cm from the olfactory target are reported in Figure 20a and Figure 20b, respectively. The chromatogram in Figure 20a shows an intense peak corresponding to HMHA, while the signal corresponding to 3MSH is weak. On the contrary, a more intense peak corresponding to 3MSH is observed in the 15 cm snow

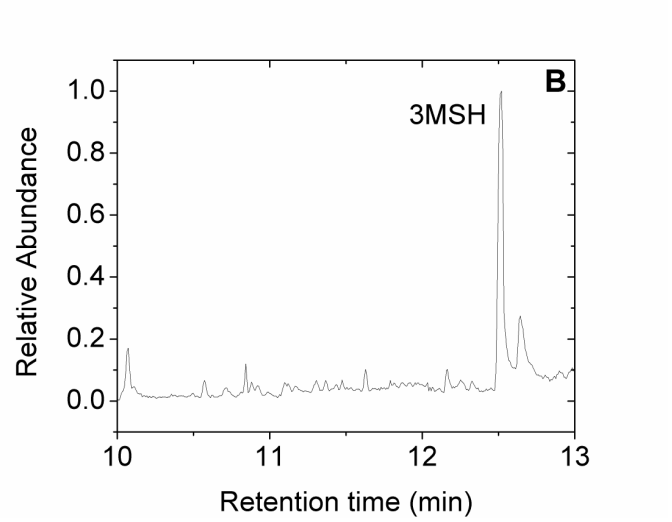
section (Figure 20b). Moreover, the matrix does not interfere with the analytes, as also suggested by the I^T/I^C ratios, which do not statistically differ from the values obtained in standard solutions.

Target ion (I^T) and control ions (I^C), see Section 4.3

The HMHA and 3MSH concentration profiles along the vertical section of the core are reported in Figure 21. As shown in Figure 21, after 20, 40, 60 and 80 min, both compounds diffuse differently through the snow. Indeed, the HMHA concentration profile fits a complementary error function(erfc)-like curve, as expected for a diffusive process when the flow rate of a flowing fluid is negligible or absent. On the contrary, the 3MSH concentration profile showed a peak-shaped profile which reaches its maximum at 10-20 cm from the target odorant. This latter profile could be due to a diffusive process in a flowing fluid medium, as occurs in chromatography [122].



(a) HMHA



(b) 3MSH

Figure 20: SIM Chromatogram obtained using DI-SPME in real samples of melted snow collected at 5 (a) and 15 cm (b) from the olfactory target.

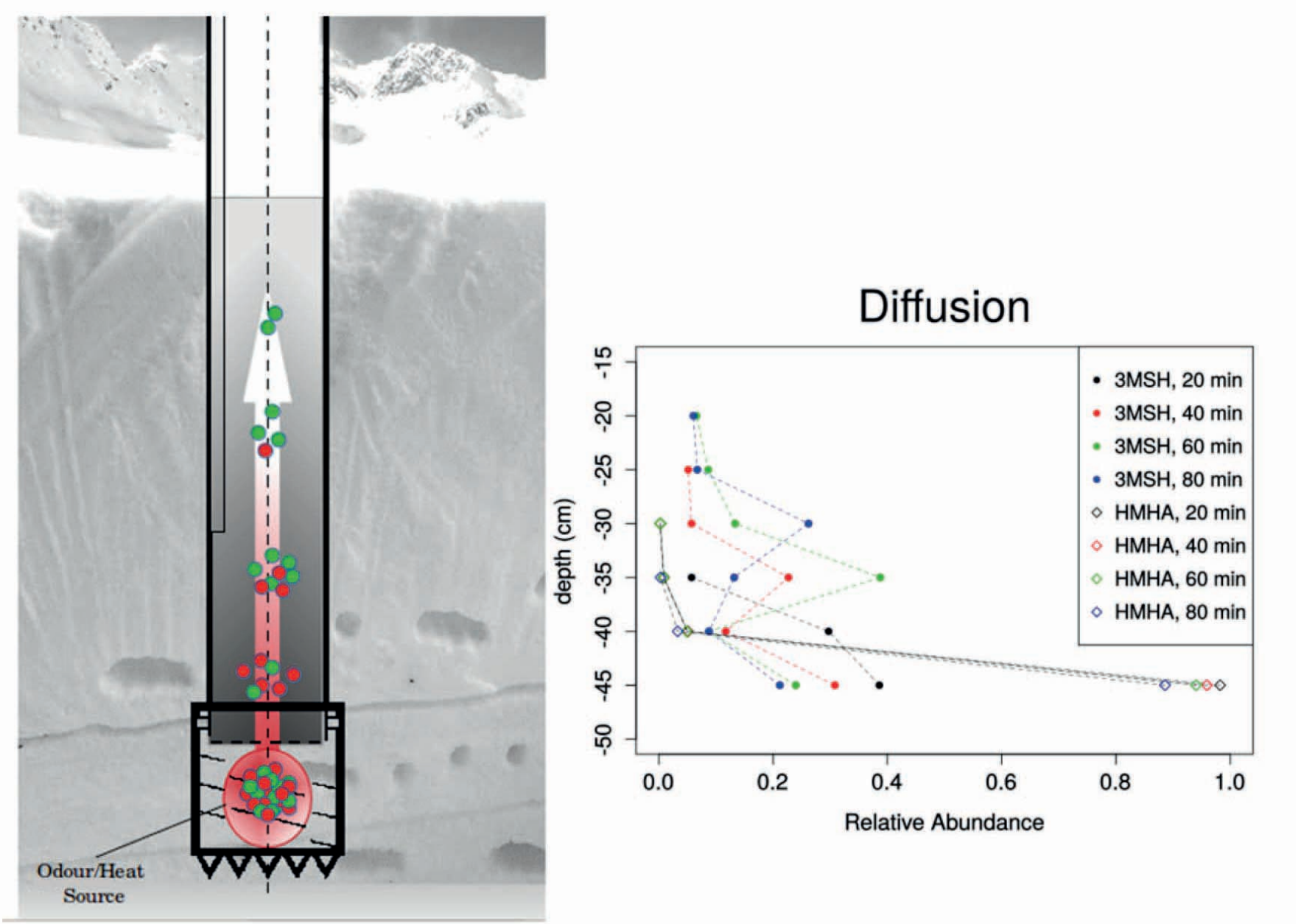


Figure 21: Figure to the left represents schematically the experimental conditions: the core barrel loaded with the snow and with the odour/heat source is placed inside in the snowpack for the specified time. Inside the core barrel are drawn two compounds that diffuse with different mechanism which determines the vertical separation of the molecules. Figure to the right shows the experimental diffusion profile of HMHA (⋄) and 3MSH (•) through 0.5 m snow column after 20, 40, 60 and 80 min.

5.4 DISCUSSION

The diffusion profiles of the two substances are different. One possible explanation could be their differencies in in hydrophilic properties, as HMHA is more hydrophilic (LogP = 1.7) compared to 3MSH (LogP = 2.14). Therefore it is likely that HMHA remains as solute in the snow melted in the immediate vicinity of the heat source while 3MSH seems to diffuse more easily through the pores of the snow as if transported by a fluid. In this case the fluid could be the water vapor moving upward due to the strong thermal gradient imposed by the presence of a heat source.

Another element that could support this hypothesis is the slowdown of the diffusion rate when the thermal gradient is decreasing. As already indicated in Figure 19 the temperature of the heat source falls drastically after few minutes, and consequently it is very likely that a greater rate of thermal diffusion is present only in the first instants of the test. If the diffusion rate were constant (and the analyte detectable), one could assume that after 80 minutes the peak of 3MSH should be approximately at 20 cm from the surface, but already after 40 minutes it appears that the diffusion induced by the thermal gradient is negligible and 3MSH stops at depth of -35 cm (Figure 21). A solution to prolong the effect of the thermal gradient may be to increase the volume of the heat source, but unfortunately this was not possible due to the reduced volume inside the corer head. We are currently studing an electronic system with a heating surface able to perform a controlled temperature decay.

5.5 CONCLUDING REMARKS

This chapter describes the method developed to study the diffusion of volatile organic compounds in the snowpack. The developed instrumentation and sampling procedures are shown here for the first time and represent a novelty in the scientific community specialised in the study of diffusion processes and VOCs accumulation in the surface snow.

We demonstrated the vertical diffusion of human odour molecules within a snowpack in the typical conditions of the site of accumulation of a slab avalanche.

Part III

CONCLUSIONS & FUTURE PERSPECTIVES

6

CONCLUSIONS

From this study emerge results of great importance as regards the development of innovative training methods for avalanche dogs through the use of synthetic molecular markers.

The main purpose of identify molecular markers of sweat was achieved with the development of specific tools and analytical methods. We were able to identify, between the large amounts of VOCs emitted, molecules specific of the body odour. In addition, to provide a reliable tool for investigating diffusion processes in snow, we developed the first anlytical method for the identification of specific components of the human odor in aqueous systems.

For the aim of training dogs to find people buried without localization tools we selected 3-Metylhex-2-enoic acid, 3-hydroxy-3-methylhexanoic acid and 3-Methyl-3-sulfanilhexanol as potential olfactory target since these compounds are gender specific and emitted by all individuals. Moreover, the development of tecniques to study the diffusion mechanism of these compounds in snow provides a solid evidence that these molecules, and in particular 3-Methyl-3-sulfanilhexanol, are suitable to be used as molecular target in replacement of the currently used olfactory target. Also preliminary test conducted with dogs indicates that we are going in the right direction.

Thanks to the help of the Guardia di Finanza and to the personal of the Avalanche Center of Arabba we were able to build a uniform snowpack with the characteristics of the snow accumulated by an avalanche. Given the complexity and the variability of the snowpack, we consider this a great success as it allowed the study of the diffusion of VOCs in a controlled environment. The results of this experiment, perhaps the most relevant results of the present work, have a great importance since the diffusion of organic compunds from the bottom to the surface of the snow driven by thermal gradient was scarcely ever reported. Finally, the outcomes of this study opens the way for further investigation to better understand the fate of volatile organic compounds in snow.

FUTURE PERSPECTIVES Looking ahead, the research will involve other substances to the molecular target pool. Some preliminary tests conducted with the dog handlers of GdF suggest to concentrate research on substances emitted from the breath and on synthetic VOCs present in sunscreens used in mountaineering (see Appendix c). Finally, an interesting investigation concerns the modeling of the diffusion of the target in the snow and the identification of environmental variables that influence the phenomenon. In this regard, we have established a contact with the SLF center of Davos which over the years has developed the Snowpack model for the mass/energy balance within the snowpack.

Part IV

APPENDIX

a

SPME BACKGROUND THEORY

A.1 SELECTION OF THE APPROPRIATE SPME FIBER

The selection of the fibers must be conducted by evaluating the molecular weight of the analytes, their polarity, their concentration and the complexity of the sample.

Smaller analytes are faster in reaching the equilibrium but are less retained with respect to the analytes of greater molecular weight that reach the equilibrium in more time. Plays an important role in the balance also the thickness of the stationary phase: indeed smaller molecules are better retained in thicker coating.

It must also take into account the shape of the analytes. For a rigid planar molecule, such as a polyene without substituents, that interacts through π - π interactions with the coating, it is observed that the molecule has extraction efficiency similar to an hydrocarbon of molecular weight 30-50 amu higher. Opposite case is observed for highly branched or aromatic molecule with electronegative substituents for which the interaction with the coating is reduced and thus the extraction efficiency appears similar to molecules of approximately 30 amu less in molecular weight.

The polarity of the sample plays an important role in the efficiency of extraction when the molecular weight of the analyte exceeds 80 amu. There are two polar fibers on the market: the 60 μm PEG and 85 μm PA. While other SPME fibers still have some affinity with polar analytes, it is desirable that the polar fibers are very selective. Consequently, fibers with polar coating are not suitable for the purposes of the tests described in this chapter.

Finally, the analyte concentration and the complexity of the matrix play an important role in the choice of fiber. Fibers that extract the analytes through an adsorption process have a lower capacity than the fibers of similar thickness that use a mechanism of absorption. Because of this, an increasing competition between the analytes for the available sites occurs, therefore the fibers with the extraction process for adsorption are not suitable for concentrated solutions. The complexity of the matrix leads to a further increase of the competition between the analytes. An analyte in greater concentration or greater affinity could mask the presence of an analyte at a lower concentration or lower affinity if the available sites are not sufficient. The use of fibers with multiple layers as the DVB - Carboxen- PDMS can in this case improve the extraction since the layers have complementary

affinity. In the case of low concentrations and times of extraction relatively short, these fibers are the most suitable.

A.2 EQUILIBRIUM EXTRACTION

The extraction equilibrium method is widely used for the SPME, especially for sampling in the environment. In this method the fiber is exposed to the sample matrix until it is reached the equilibrium, the conditions of which are described by the Equation 5, in accordance with the law of conservation of mass, if we consider only two phases (fiber and sample matrix):

$$n = \frac{K_{fs} V_f V_s}{K_{fs} V_f + V_s} C_0 \tag{5}$$

In the Equation 5 C_0 is the initial concetrazione of the target in the sample, V_s is the sample volume, V_f is the volume of the fiber cladding and K_{fs} is the distribution coefficient of the analytes between the fiber coating and the sample. The Equation 5 indicates that the amount of analyte extracted in the coating (n) is linearly proportional to concetrazione of the analyte in the sample. The equation is also the theoretical basis for a quantitative study via SPME[60].

When the sample volume is very large, as is the case of environment analysis, $V_s >> K_{fs} V_f$ and the equation is simplified:

$$n = K_{fs} V_f C_0 \tag{6}$$

It seems clear, therefore, the advantage of using the method of extraction equilibrium. In accordance with the Equation 6, the equilibrium amount of analyte extracted into the fiber is independent of the volume of the sample. The Equation 6 then allows us to analyze a sample without taking account of its volume and the fiber can be exposed directly to air, water, etc. In this case the amount of analyte extracted directly correspond to its concentration without depending on the volume. This greatly simplifies the sampling and accelerates the process of analysis by reducing the number of variables to consider. A last very important feature of equilibrium extraction is the ability to determine the concentration of analyte in the sample if it is known the distribution coefficient of the analyte between the fiber and the matrix. There are numerous works in the literature for the experimental measurement of this coefficient[60].

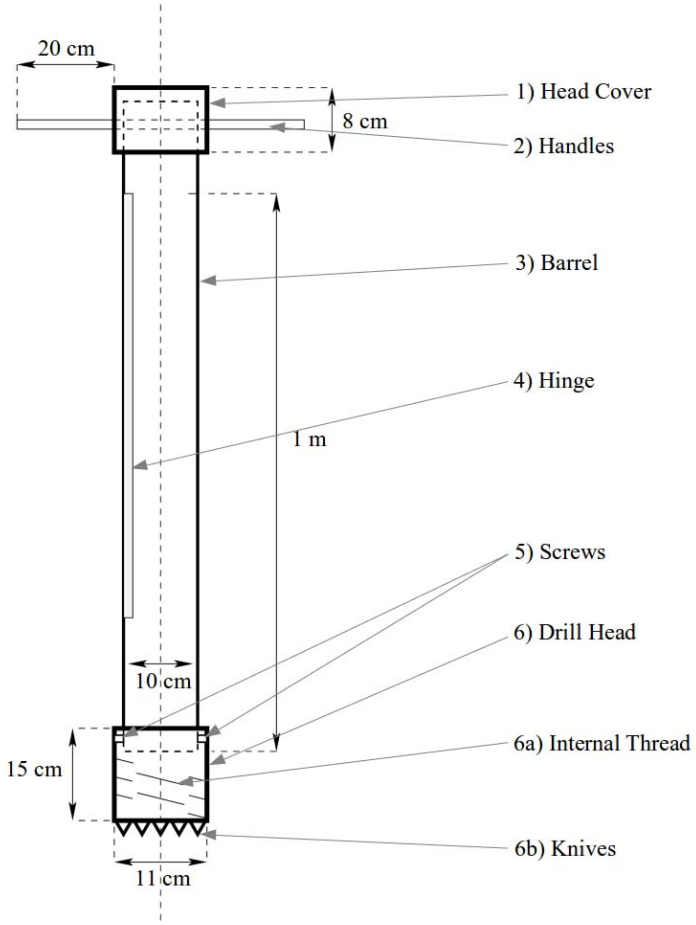


Figure 22: Snow corer, designed and developed in the mechanical laboratory of the Ca'Foscari University. It allows sampling up to 1 m of superficial snow and permits the direct access to the inner snow, preserving the physical structure of the snowpack layers.

The corer is used to sample up to a meter of superficial snow and allows access to various parts of the snow removed preserving the physical structure of the layers of snow.

1) The head cover, made of Teflon, is the upper seat of the barrel. It is a cylinder of 8 cm in height, an internal diameter of 10.2 cm and an external diameter of 11 cm. Head cover and barrel are fixed

through two diametrically opposite holes on which the handles are inserted.

2) The handles are formed by a stainless steel bar length of about 51 cm and a diameter of 2.5 cm.

3) The barrel is made from a stainless steel cylinder of 1.20 m length, inner diameter of 10 cm and an outer diameter of 10.2 cm. The barrel is cut for the entire length so as to obtain two symmetrical pieces. A hinge allows opening the barrel to access the core drilling.

4) The stainless steel hinge is 60 cm long and is fixed to the barrel so as to allow its opening.

5) Four stainless steel screws secure the barrel at the drill head.

6) the drill head is a stainless steel cylinder 15 cm long, internal diameter of 10.2 cm and an outer diameter of 11 cm. The interior of the drill head is threaded
   a) with a screw pitch of 10 cm and the thickness of the thread is 2 cm. One end of the drill head is fixed to the barrel through the screws, while the opposite end is equipped with knives
   b) for cutting the lenses of ice possibly present in the snowpack.

SNOW SAMPLING

step 1) Place the corer upright on the snow and start to screw using the handles. The corer will start to penetrate into the snow and the thread of the drill head will allow to measure the depth reached (Figure 18a).

step 2) Upon reaching the desired depth, stop screwing and slowly pull the corer because a fast extraction of the corer can damage the layers of snow inside the barrel.

step 3) Once extracted the corer from the snow and laid on a flat surface, release the barrel by pulling the handles, remove the cover and the drill head once unscrewed (Figure 18b).

step 4) Remove the snow from the drill head and put the heat source inside the drill head (Figure 18c). The heat source consists of a thermos filled with water at 37 ° and with an internal thermometer to monitor the temperature change. In order not to spill the water, the thermos it is wrapped with a polyethylene film. Above the heat source is placed the sample (HMHA and 3MSH) which consists of a disk of cotton soaked (Figure 18d,Figure 18e).

step 5) Place the barrel on the drill head (Figure 18f) and prevent the escape of volatile sample wrapping the core barrel with a polyethylene film.

step 6) Secure the screws and now insert the core barrel into the hole. Then cover the outer parts of the core barrel with snow taken in the vicinity of the sampling site (Figure 18g).

step 7) Extract the corer from the snow and access the snow sampled by opening the corer (Figure 18g).

C

BREATHING SYSTEM

To simulate the condition of a people buried under an avalanche, we developed an original system that allows to breath under the snow in controlled environment. We already obtained some important results with the dogs that showed a strong interest in the scent of breath (Figure 26). We present here, through some photographs, the major characteristics of the experiment since it represents the future of the project.

Figure 23: Late in 2015, before winter snowfalls, we fixed a system of PVC tubes to the ground. One end of the tube is open in the atmosphere, the other end is protected inside a stainless steel gas diffuser of about 1.2 dm^3 that simulates the air pocket available to the buried person. Diffusers, designed with the personal of the Guardia di Finanza and produced by the technicians of SAGF, protect the end of the tubes from ice sealing.

Figure 24: We organized the experimental site as to allow several tests. During winter and spring we monitored the snowpack, the environmental condition and checked the ice formation on the diffuser.

Figure 25: We simulated breathing inside the snowpack. We did not considered yet the use of a mask to completely isolates the volunteer's face since our main concern was the ice formation and to study the condition of the snow after the test. All tests were positive and the next year we will collect snow samples to study the compounds emitted through the breath. (In the picture: SAGF instructor Walter Levis.)

Figure 26: The experiment conducted with avalanche dogs. A plastic pipe was buried under the snow, whose characteristics were similar to those of an avalanche. A volunteer breathed in the snowpack. In all the tests conducted dogs have found the end of the tube hidden under the snow. These pictures are property of Costanza Azzari.

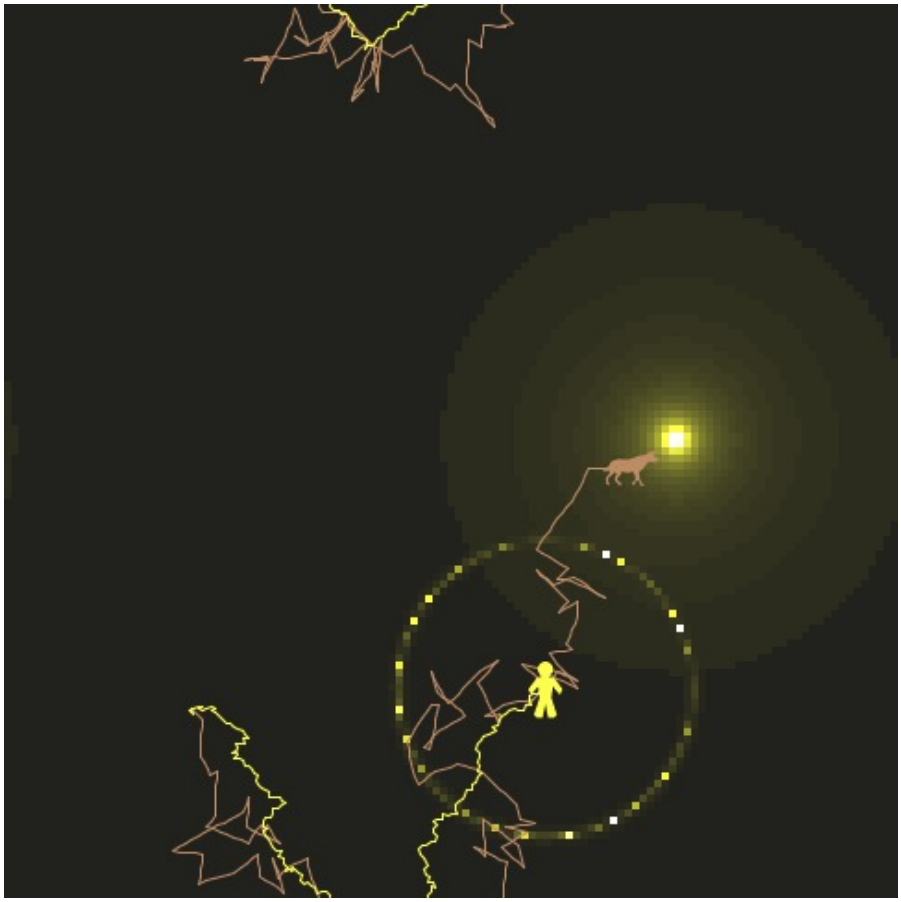


Figure 27: Image of the program that simulates the research of the canine units in avalanche.

We present here the features of a program we developed with NetLogo program. Although not directly connected with the study of VOCs this program will be developed, in collaboration with the instructors of the SAGF and with the veterinarians of University of Perugia, in order to describe *the search* by the canine unit with a global model that takes into account environmental variables, diffusion of odors in the snowpack and the character features of the canine unit.

There are three subject: the force field, the dog and the dog handler. The force field simulates the presence of something that is of interests for the dog. The greater is the intensity (yellow), the greater is the level of interest for the dog, there can be one or more *targets*. The dog handler also produces a force field around himself and the strenght of the dog handler's field is proportional to his personality.

The dog handler always moves in the direction of the position of the dog. Finally the dog is able to feel the force field and tend to move toward the the target (where the field is stronger).

At the beginning of the software simulation, the position of the buried person and the canine unit is randomly chosen. The simulation end when both the dog and the dog handler arrives above the target.

Through this simple model we can already describe many aspect of the research by the canine units. In our view could be interesting to compare the simulated patterns given by this simple model with the real patterns showed by the canine units during the search for the buried person.

e

MOLECULES EMITTED BY HUMAN BODY

The Table 7 cover as much as possible the large variety of VOCs that are cited in literature. Are reported the name of the compound, CAS number, the molecular class, weight, body source and finally the bibliographic reference.

Table 7: Quì mettiamo la didascalia.

COMPOUNDS	#CAS	CLASS	PM	HUMAN SOURCE	REFERENCES
Carboxylic acids					
Methanoic acid (Formic acid)	64-18-6	C1	46.03	Urine	[123]
Acetic acid	64-19-7	C2	60.05	Sweat/Urine	[35, 47, 76, 123, 124]
Propanoic acid	79-09-4	C3	74.08	Sweat	[35, 76]
2-Hydroxypropanoique acid (lactic acid)	50-21-5	C3	90.08	Sweat	[71]
3-Hydroxybutyric acid	300-85-6	C4	104.10	Urine	[125]
2-Methylpropanoic acid (isobutanoic acid)	79-31-2	C4	88.10	Sweat	[76]
Butanoic acid (butyric acid)	107-92-6	C4	88.11	Sweat/Urine	[35, 47, 76, 123]
2-Methylbutanoic acid (isovaleric acid)	503-74-2	C5	102.12	Sweat/Urine	[35, 76, 81, 123]
Pentanoic acid	109-52-4	C5	102.13	Sweat	[76]
3-Hydroxyhexanoic acid	10191-24-9	C6	132.15	Sweat	[77]
4-Methylpentanoic acid (isohexanoic acid)	646-07-1	C6	115.15	Sweat	[76]
3-Methyl-2-oxopentanoic acid	1460-34-0	C6	130.14	Sweat	[71]
4-Methyl-2-oxopentanoic acid	816-66-0	C6	130.14	Sweat	[71]
(E)-3-Methyl-2-pentenoic acid	16957-70-3	C6	114.14	Sweat	[39, 126]

COMPOUNDS	#CAS	CLASS	PM	HUMAN SOURCE	REFERENCES
Hexanoic acid	142-62-1	C6	116.16	Sweat	[35, 38, 39, 126]
(Z)-4-Methylhex-3-enoic acid	55665-79-7	C7	128.17	Sweat	[82]
3-Hydroxyheptanoic acid	17587-29-0	C7	146.18	Sweat	[77]
3-Hydroxy-4-methylhexanoic acid	59866-91-0	C7	146.18	Sweat	[71, 77]
(R)/(S)-3-Hydroxy-3-methylhexanoic acid	58888-76-9	C7	146.18	Sweat	[71, 77, 82, 127]
2-Methylhexanoic acid	4536-23-6	C7	129.17	Sweat	[38, 39]
Heptanoic acid	111-14-8	C7	129.17	Sweat	[38, 39]
4-Ethylpentanoic acid	1561-11-1	C7	130.18	Sweat	[38, 39]
3-Methylhexanoic acid	3780-58-3	C7	130.18	Sweat	[38, 126]
(Z)-3-Methylhex-2-enoic acid	54068-86-9	C7	128.16	Sweat	[38, 39, 71, 77, 78, 82, 126, 127]
(E)-3-Methylhex-2-enoic acid	27960-21-0	C7	128.16	Sweat	[38, 39, 71, 77, 78, 82, 126, 127]
(4-Hydroxyphenyl)acetic acid	156-38-7	C8	152.15	Sweat	[71]
Octanedioic acid (suberic acid)	505-48-6	C8	174.20	Sweat	[71]
3-Hydroxy-3-methylheptanoic acid	160595-71-1	C8	160.21	Sweat	[71, 77]
3-Hydroxyoctanoic acid	14292-27-4	C8	160.21	Sweat	[71, 77]
Phenylacetic acid (benzeneacetic acid)	103-82-2	C8	136.14	Sweat	[71, 77]
8-Hydroxyoctanoic acid	764-89-6	C8	160.21	Sweat	[71, 77]

COMPOUNDS	#CAS	CLASS	PM	HUMAN SOURCE	REFERENCES
Octanoic acid	124-07-2	C8	144.21	Sweat/Urine	[38, 123, 126]
3-Hydroxy-4-methylheptanoic acid	903503-32-2	C8	160.21	Sweat	[71, 77, 81]
2-Methylheptanoic acid	1188-02-9	C8	143.20	Sweat	[38, 39]
Octanoic acid	124-07-2	C8	144.21	Sweat	[38, 39]
2-Ethylhexanoic acid	149-57-5	C8	144.21	Sweat	[35, 38, 39]
7-Octenoic acid	18719-24-9	C8	142.20	Sweat	[38, 39]
9-Hydroxynonanoic acid	3788-56-5	C9	174.24	Sweat	[71]
Nonanedioic acid (azelaic acid)	123-99-9	C9	188.22	Sweat	[71]
(E/Z)-4-Methyloct-3-enoic acid	931088-73-2	C9	156.00	Sweat	[71, 77]
3-Hydroxy-3-methyloctanoic acid	6966-34-3	C9	174.23	Sweat	[71, 77]
3-Hydroxy-4-methyloctanoic acid	875712-96-2	C9	174.23	Sweat	[71, 77]
9-Hydroxynonanoic acid	3788-56-5	C9	174.24	Sweat	[71, 77]
(E)-3-Methyl-2-octenoic acid	90646-67-6	C9	156.22	Sweat	[39]
2-Methyloctanoic acid	3004-93-1	C9	158.23	Sweat	[38, 39]
4-Ethylheptanoic acid	132735-95-6	C9	158.24	Sweat	[38, 39, 71]
Nonanoic acid	112-05-0	C9	158.23	Sweat/Urine	[38, 39, 123, 126]
3-Hydroxy-4-methylnonanoic acid	903503-33-3	C10	158.28	Sweat	[71, 77]

COMPOUNDS	#CAS	CLASS	PM	HUMAN SOURCE	REFERENCES
3-Hydroxydecanoic acid	14292-26-3	C10	158.28	Sweat	[71, 77]
(E/Z)-4-Methylnon-3-enoic acid	61271-90-7	C10	170.24	Sweat	[71]
2-Methylnonanoic acid	24323-21-5	C10	172.26	Sweat	[38, 39]
9-Decenoic acid	14436-32-9	C10	170.25	Sweat	[38, 39]
4-Ethyloctanoic acid (goat acid)	16493-80-4	C10	18.60	Sweat	[38, 39, 71, 77]
Decanoic acid	334-48-5	C10	172.26	Sweat	[38, 39]
10-Undecenoic acid	112-38-9	C11	184.28	Sweat	[39]
(R/S)-3-Hydroxy-4-methyldecanoic acid	24323-23-7	C11	186.29	Sweat	[77, 126]
2-Methyldecanoic acid	24323-23-7	C11	186.29	Sweat	[38, 39]
4-Ethylnonanoic acid	137168-02-6	C11	186.29	Sweat	[38, 39]
Undecanoic acid	112-37-8	C11	186.29	Sweat	[38, 39, 126]
Dodecanoic acid	143-07-7	C12	200.32	Sweat	[34]
4-Ethyldecanoic acid	25234-31-5	C12	200.32	Sweat	[38, 39]
Tetradecanoic acid	544-63-8	C14	228.37	Sweat	[34]
Alcohols					
Methanol	67-56-1	C1	32.04	Breath	[128]
Ethanol (ethyl alcohol)	64-17-5	C2	46.06	Breath/Urine	[58, 128, 129]

COMPOUNDS	#CAS	CLASS	PM	HUMAN SOURCE	REFERENCES
1-Propanol	71-23-8	C3	60.10	Breath	[130]
2-Propanol	67-63-0	C3	60.10	Breath	[128]
1-Butanol	71-36-3	C4	74.12	Breath/Sweat/Urine	[47, 123, 130]
3-Methyl-3-buten-1-ol	763-32-6	C5	86.13	Urine	[123]
2-furanmethanol (furfuryl alcohol)	98-00-0	C5	98.10	Sweat/Urine	[34, 35, 123]
2-Methyl-butan-1-ol	137-32-6	C5	88.15	Sweat	[78]
1-Pentanol	71-41-0	C5	88.14	Sweat	[47]
3-methyl-1-butanol (isopentanol)	123-51-3	C5	88.14	Urine	[80]
Cyclohexanol	108-93-0	C6	100.15	Urine	[80]
1,1'-oxybis-2-propanol	110-98-5	C6	134.17	Sweat	[35]
1-Hexanol	111-27-3	C6	102.17	Sweat/Urine	[47, 123]
Phenol	108-95-2	C6	94.11	Sweat/Urine	[34, 35, 38, 39, 80, 123]
5-Methyl-3-hexanol	623-55-2	C7	116.20	Urine	[123]
4-Methylphenol (p-cresol)	106-44-5	C7	108.13	Urine	[80]
1-(2-methoxypropoxy)-2-propanol	1321-21-7	C7	148.20	Sweat	[35]
Benzyl alcohol (phenylmethanol)	100-51-6	C7	108.14	Sweat/Urine	[123]
3-Methyl-1-hexanol	13231-81-7	C7	116.20	Sweat	

COMPOUNDS	#CAS	CLASS	PM	HUMAN SOURCE	REFERENCES
1-Octen-3-ol	3391-86-4	C8	128.21	Sweat	[35]
2-Phenylethanol	60-12-8	C8	122.16	Sweat	[35]
Octanol	111-87-5	C8	130.22	Sweat	[47]
2-Ethyl-1-hexanol	104-76-7	C8	130.22	Breath/Sweat/Urine	[35, 47, 83]
3,7-dimethyl-1,6-octadien-3-ol (Linalool)	78-70-6	C10	154.25	Sweat/Urine	[35, 80]
2,6-Dimethyl-7-octen-2-ol (dihydromyrcenol)	18479-58-8	C10	156.27	Sweat	[35]
2-(2-propyl)-5-methyl-1-cyclohexanol (menthol)	89-78-1	C10	156.27	Sweat	[35]
3,7-dimethyl-6-octen-1-ol (citronellol)	106-22-9	C10	156.27	Sweat	[35]
3,7-dimethyl-6-octadien-1-ol (geraniol)	106-24-1	C10	154.25	Sweat	[35]
1-Decanol	112-30-1	C10	158.28	Sweat	[47]
Tetradecanol	112-72-1	C14	214.39	Sweat	[38, 39]
1-Hydroxypyrene	5315-79-7	C16	218.25	Urine	[131]
Hexadecanol	36653-82-4	C16	242.44	Sweat	[38, 47]
2,6-bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	14035-34-8	C17	262.39	Sweat	[35]
2,4,6-tri-tert-butyl-phenol	732-26-3	C18	262.43	Sweat	[35]
Octadecanol	112-92-5	C18	270.49	Sweat	[47]

COMPOUNDS	#CAS	CLASS	PM	HUMAN SOURCE	REFERENCES
Aldehydes					
Methanal (formaldehyde)	50-00-0	C1	30.03	Urine	[132]
Ethanal (acetaldehyde)	75-07-0	C2	44.05	Breath	[58, 128, 129]
Propanal (propionaldehyde)	123-38-6	C3	58.07	Urine	[133]
2-Methylpropanal (Isobutanal)	78-84-2	C4	72.10	Urine	[133]
2-Methylbutanal	96-17-3	C5	86.13	Breath/Urine	[133]
2-Methyl-2-butenal	497-03-0	C5	84.11	Urine	[133]
Pentanal	110-62-3	C5	86.13	Urine	[133]
2-furancarboxaldehyde (furfural)	98-01-1	C5	96.08	Sweat	[34]
Hexanal	66-25-1	C6	100.15	Breath/Sweat/Urine	[34, 47, 123, 133]
Benzaldehyde	100-52-7	C7	106.12	Sweat/Urine	[34, 35, 123]
Heptanal	111-71-7	C7	114.19	Sweat	[34, 47]
Octanal	124-13-0	C8	128.21	Breath/Sweat/Urine	[34, 35, 47, 58, 133]
Nonanal	124-19-6	C9	142.23	Breath/Sweat	[35, 47, 130]
(E)/(Z)-2-Nonenal	18829-56-6	C9	142.23	Sweat	[34, 47]
Decanal (Decyl aldehyde)	112-31-2	C10	156.26	Sweat	[47]
Undecanal	112-44-7	C11	170.29	Sweat	[34]

COMPOUNDS	#CAS	CLASS	PM	HUMAN SOURCE	REFERENCES
Dodecanal	112-54-9	C12	184.32	Sweat	[35]
Tetradecanal	124-25-4	C14	212.37	Sweat	[34]
Esters					
Methyl acetate	79-20-9	C3	74.08	Urine	[134]
Ethyl acetate	141-78-6	C4	88.11	Urine	[123, 134]
Dimethyl malonate	108-59-8	C5	132.11	Sweat	[34]
Methyl 2-furoate	611-13-2	C6	126.11	Sweat	[34]
Methyl hexanoate	106-70-7	C7	130.18	Sweat	[34]
Butyl butanoate	109-21-7	C8	144.21	Urine	[123]
Hexanedioic acid dimethyl ester (dimethyl hexanedioate)	627-93-0	C8	174.19	Sweat	[34]
Methyl octanoate	111-11-5	C9	158.23	Sweat	[34]
Phenylmethyl acetate (benzyl acetate)	140-11-4	C9	150.18	Sweat	[34]
Ethyl phenylacetate	101-97-3	C10	164.20	Sweat	[35]
Methyl nonanoate	1731-84-6	C10	172.26	Sweat	[34]
Methyl decanoate	110-42-9	C11	186.29	Sweat	[34]
4-tert-butylcyclohexyl acetate (vertnex)	32210-23-4	C12	198.30	Sweat	[35]
Methyl undecanoate	1731-86-8	C12	200.32	Sweat	[34]

COMPOUNDS	#CAS	CLASS	PM	HUMAN SOURCE	REFERENCES
Methyl dodecanoate	111-82-0	C13	214.34	Sweat	[34]
Methyl tridecanoate	1731-88-0	C14	228.37	Sweat	[34]
10-Methyl dodecanoic acid methyl ester	5129-65-7	C14	228.37	Sweat	[34]
Methyl tetradecanoate	124-10-7	C14	242.40	Sweat	[34]
2-Ethylhexyl 2-ethylhexanoate	7425-14-1	C16	256.48	Sweat	[35]
9-Methyltetradecanoic acid methyl ester	213617-69-7	C16	256.42	Sweat	[34]
Methyl pentadecanoate	7132-64-1	C16	256.42	Sweat	[34]
Tetradecanoic acid, 1-methylethyl ester	110-27-0	C17	270.45	Sweat	[35]
Methyl hexadecanoate	112-39-0	C17	270.45	Sweat	[34]
7-Hexadecenoic acid methyl ester	93479-57-3	C17	268.43	Sweat	[34]
Cyclopentane tridecanoic acid methyl ester	24828-61-3	C18	296.49	Sweat	[34]
Halogenated					
Chloroform	67-66-3	C1	119.38	Urine	[80]
Chloromethane	74-87-3	C1	50.48	Breath	[135]
Tetrachloroethylene	127-18-4	C2	165.85	Urine	[80]
Chlorocyclohexane	542-18-7	C6	118.60	Urine	[80]
1,4-Dichlorobenzene	106-46-7	C6	147.00	Urine	[80]

COMPOUNDS	#CAS	CLASS	PM	HUMAN SOURCE	REFERENCES
1-Chlorononane	2473-01-0	C9	162.7	Sweat	[34]
Heterocyclic					
Furan	110-00-9	C4	68.07	Urine	[135]
Pyrazine	290-37-9	C4	80.09	Urine	[123]
1H-Pyrrole	109-97-7	C4	67.09	Urine	[123]
2-Methylpyrazine	109-08-0	C5	94.11	Urine	[123]
Piperidine	110-89-4	C5	85.15	Urine	[123]
1-Methylpyrrole	96-54-8	C5	81.12	Urine	[123]
2-Methylfuran	534-22-5	C5	82.10	Urine	[123]
Pyridine	110-86-1	C5	79.09	Sweat/Urine	[123]
2-Piperidinone	675-20-7	C5	99.13	Sweat	[39]
2,5-Dimethylpyrazine	123-32-0	C6	108.14	Urine	[123]
1-Methyl-2-piperidinone	931-20-4	C6	113.16	Urine	[123]
3-Methylpyridine	108-99-6	C6	93.13	Urine	[123]
2-Vinylpyrazine	4177-16-6	C6	106.13	Urine	[123]
2-Acetylfuran	1192-62-7	C6	110.11	Urine	[123]
2,5 Dimethylfuran	625-86-5	C6	96.13	Urine	[123]

COMPOUNDS	#CAS	CLASS	PM	HUMAN SOURCE	REFERENCES
2-Ethyl-5-methylfuran	1703-52-2	C7	110.15	Urine	[123]
Hydrocarbons					
Ethane	74-84-0	C2	30.06	Breath	[125]
Pentane	109-66-0	C5	72.14	Breath	[125]
1,2-Pentadiene	591-95-7	C5	68.12	Breath	[135]
2-Methylbut-1,3-diene (isoprene)	78-79-5	C5	68.11	Breath	[128, 129, 133, 135]
Cyclohexene	110-83-8	C6	82.14	Urine	[80]
Benzene	71-43-2	C6	78.11	Breath/Urine	[58, 80]
Hexane	110-54-3	C6	86.18	Breath	[136]
2-Methyl-1-pentene	763-29-1	C6	84.16	Breath	[79]
2-Methylpentane	107-83-5	C6	86.17	Breath	[79, 137]
Toluene (methylbenzene)	108-88-3	C7	92.14	Sweat/Urine	[34]
2-Methylhexane	591-76-4	C7	100.20	Breath	[137]
3-Methylhexane	589-34-4	C7	100.20	Breath	[137]
Ethyl benzene	100-41-4	C8	106.16	Urine	[138]
m-Xylene	108-38-3	C8	106.16	Urine	[138]
o-Xylene	95-47-6	C8	106.16	Urine	[138]

COMPOUNDS	#CAS	CLASS	PM	HUMAN SOURCE	REFERENCES
2,2-Dimethylhexane	590-73-8	C8	114.22	Urine	[58]
p-Xylene (1,4-dimethylbenzene)	106-42-3	C8	106.16	Urine	[58, 138]
1-Octene	111-66-0	C8	112.24	Sweat	[47]
2-Methylheptane	592-27-8	C8	114.23	Breath	[58]
2,3-Dimethylhexane	584-94-1	C8	114.22	Breath	[58]
1,2,3-Trimethylbenzene	526-73-8	C9	120.19	Urine	[139]
Nonane	111-84-2	C9	128.26	Sweat	[34]
α-Methylstyrene	98-83-9	C9	118.18	Breath	[130]
2,4-Dimethylheptane	2213-23-2	C9	128.26	Breath	[130, 134]
1-Methyl-2-(1-methylethyl)benzene	527-84-4	C10	134.21	Urine	[80]
Naphtalene	91-20-3	C10	128.17	Sweat	[34]
Decane	124-18-5	C10	142.28	Sweat	[47]
Undecane	1120-21-4	C11	156.31	Sweat	[47]
Dodecane	112-40-3	C12	170.33	Sweat	[47]
Tridecane	629-50-5	C13	184.36	Sweat	[34]
Tetradecane	629-59-4	C14	198.39	Sweat	[34]
Hexadecane	544-76-3	C16	226.42	Breath/Sweat	[34]

COMPOUNDS	#CAS	CLASS	PM	HUMAN SOURCE	REFERENCES
Heptadecane	629-78-7	C17	240.47	Sweat	[34]
Ketones					
Acetone	67-64-1	C3	58.07	Breath/Sweat/Urine	[35, 123]
2,3-Butanedione (diacetyl)	431-03-8	C4	86.09	Urine	[134]
2-Butanone	78-93-3	C4	72.11	Urine	[123]
2-Methylbutanone	563-80-4	C5	86.13	Urine	[134]
Cyclopentanone	120-92-3	C5	84.11	Urine	[133]
3-Methyl-2-butanone	563-80-4	C5	86.13	Urine	[133]
2-Pentanone	107-87-9	C5	86.13	Urine	[123]
3-Penten-2-one	625-33-2	C5	84.12	Urine	[80]
3-Methylcyclopentanone	1757-42-2	C6	98.14	Urine	[133]
2-Hexanone	591-78-6	C6	100.15	Urine	[125]
2-Methyl-2-cyclopentene-1-one	1120-73-6	C6	96.13	Urine	[123]
4-Methyl-3-pentene-2-one	141-79-7	C6	98.14	Urine	[123]
3-Methyl-2-pentanone	565-61-7	C6	100.16	Urine	[123]
3-Hexanone	63072-44-6	C6	100.15	Urine	[123]
4-Methyl-2-pentanone	108-10-1	C6	100.16	Sweat/Urine	[47, 123]

COMPOUNDS	#CAS	CLASS	PM	HUMAN SOURCE	REFERENCES
3-Methylcyclohexanone	591-24-2	C7	112.17	Urine	[123]
3-Dimethyl-2-cyclo-pentene-1-one	1121-05-7	C7	110.15	Urine	[123]
3-Methyl-2-hexanone	2550-21-2	C7	114.19	Urine	[80]
3-Heptanone	106-35-4	C7	114.19	Urine	[123]
2-Heptanone	110-43-0	C7	114.19	Urine	[123]
4-Heptanone	123-19-3	C7	114.19	Urine	[58, 80]
3-Methyl-2-heptanone	2371-19-9	C8	128.21	Urine	[80]
Acetophenone	98-86-2	C8	120.15	Sweat	[35]
6-Methyl-5-hepten-2-one (prenylacetone)	110-93-0	C8	126.20	Sweat	[35]
6-Methyl-5-heptanone (2-methyl-3-heptanone)	13019-20-0	C8	128.21	Sweat	[35, 47]
3,5,5-Trimethylcyclohex-2-en-1-one (isophorone)	78-59-1	C9	138.21	Sweat	[35]
2-Decanone	693-54-9	C10	156.23	Urine	[80]
1,7,7-trimethylbicyclo[2,2,1]heptan-2-one (camphor)	76-22-2	C10	152.23	Sweat	[35]
(Z)-6,10-Dimethyl-5,9-undecadien-2-one	3879-26-3	C13	194.31	Sweat/Urine	[35]
(E)-6,10-Dimethyl-5,9-undecadien-2-one (geranylacetone)	3796-70-1	C13	194.31	Sweat/Urine	[35]
2,6-Bis(1,1-dimethylethyl)2,5-cyclohexadiene-1,4-dione	719-22-2	C14	220.31	Urine	[80]

COMPOUNDS	#CAS	CLASS	PM	HUMAN SOURCE	REFERENCES
Nitrogenated					
Ammonia	7664-41-7	/	17.03	Breath	[128, 129]
Cyanhydric acid	74-90-8	C1	27.02	Breath	[140]
Dimethylamine	124-40-3	C2	45.08	Breath/Urine	[123]
Trimethylamine	75-50-3	C3	59.11	Breath/Urine	[123]
(E)-2-Methyl-2-butenenitrile (2-cyano-2-butene)	30574-97-1	C5	81.12	Urine	[123]
4-Cyanocyclohexene	100-45-8	C7	107.15	Sweat	[35]
Sulfurated					
Methanethiol (methylmercaptan)	74-93-1	C1	48.11	Breath/Urine	[123]
Dimethylsulfoxide	67-68-5	C2	78.13	Urine	[38]
Dimethylsulfide (methylthiomethane)	75-18-3	C2	62.13	Urine	[141]
Dimethyltrisulfide	3658-80-8	C2	126.26	Urine	[123]
Dimethyldisulfide	624-92-0	C2	94.20	Urine	[123]
Dimethylsulfone	67-71-0	C2	94.13	Sweat/Urine	[35]
Ethanethiol	75-08-1	C2	62.13	Breath	[128]
5-Methylisothiazole	693-97-0	C4	99.15	Urine	[123]
Methylpropyldisulfide	2179-60-4	C4	122.25	Urine	[123]

COMPOUNDS	#CAS	CLASS	PM	HUMAN SOURCE	REFERENCES
1,3-Dithiacyclohexene	290-24-4	C4	98.70	Urine	[123]
Methyl-2-propenyldisulfide	2179-58-0	C4	120.23	Urine	[123]
3-Isothiocyanato-1-propene (allylisothiocyanate)	57-06-7	C4	99.15	Urine	[123]
2-Methylfuran-3-thiol	28588-74-1	C5	114.17	Urine	[123]
2-methyl-3-sulfanylbutan-1-ol	227456-33-9	C5	120.21	Sweat	[71, 81]
3-sulfanyl-pentan-1-ol	548740-99-4	C5	120.21	Sweat	[84]
2,4-Dimethylthiophene	638-00-6	C6	112.19	Urine	[80]
3-sulfanylhexan-1-ol	51755-83-0	C6	134.23	Sweat	[71, 81]
Isothiocyanocyclohexane	1122-82-3	C7	141.23	Urine	[133]
3-Methyl-3-sulfanylhexan-1-ol	307964-23-4	C7	148.26	Sweat	[71, 81]
Terpenes					
Carvone	2244-16-8	C10	150.22	Urine	[130]
α-Phellandrene	99-83-2	C10	136.24	Urine	[130]
α-Terpinene	99-86-5	C10	136.23	Urine	[130]
α-Terpinolene	586-62-9	C10	136.23	Urine	[130]
p-Cymene	99-87-6	C10	134.22	Sweat	[35]
α-Terpineol	98-55-5	C10	154.25	Sweat	[35]

COMPOUNDS	#CAS	CLASS	PM	HUMAN SOURCE	REFERENCES
Limonene	5989-27-5	C10	136.23	Breath	[79]
Gases					
Nitric oxide	10102-43-9	/	30.01	Breath	[128]
Carbon monoxide	630-08-0	C1	28.01	Breath	[142]
Carbon dioxide	124-38-9	C1	44.01	Breath	[142]

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Federico Dallo

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