University of South Bohemia in České Budějovice,
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Human Exposure to Allergens from Household Dust

Bachelor Thesis

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Biological Chemistry

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Bachelor Thesis

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Annotation

House dust is a significant source of human´s exposure not only to certain chemical contaminants such as pesticides, flame retardants and plasticizers but also to house dust mites and moulds which are regarded as potential health hazards. In this work, guanine which is the major nitrogenous waste product of mites was detected and quantified in several house dust samples. The results of liquid chromatography and gas chromatography determination were compared with the Acarex-test. Ergosterol was quantified in the house dust samples as it serves as a bio indicator for fungi and moulds.

Affirmation

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1 Introduction

House dust is a heterogeneous mixture composed of biologically derived materials such as skin, hair, mites and fungus spores, insect parts as well as particulate matter caused by cooking and heating, cigarette smoke, and soil brought in by footwear.[1] Indoor dust can be regarded as a significant exposure pathway for humans to chemical contaminants such as pesticides, flame retardants, plasticizers and heavy metals.[2] The levels of contaminants are often higher in indoor environments than compared to outdoor environments because many of them are absorbed by particulate matter that is initially suspended in the air and later settles as dust.[3] Therefore house dust is of big concern for human health especially for children and toddlers which have a higher uptake level of pollutants due to non-dietary ingestion of house dust.[4] Moreover, house dust mites are another harmful factor due to their production of allergy causing substances. The main diseases house dust mite allergens cause are rhinitis, asthma and atopic dermatitis. Many people are not aware of the fact that house dust mites living in our homes can be pathogenic to humans.[5]

This bachelor thesis is focused on allergenicity of house dust, which could contain guanine as the major nitrogenous waste product of mites; therefore guanine content could be used as a bio indicator of their presence. The presence of mould and fungi could be evaluated from the content of ergosterol. The main goal of the bachelor thesis is to search for the literature dealing with above mentioned allergy problems and perform basic laboratory experiments for determination of guanine and ergosterol and if possible, to compare the results with the results of the Acarex-test.

2 House Dust Mites

2.1 General information

All information about house dust mites and house dust allergy are taken out of the book „Hausstauballergien: Gesundheitliche und hygienische Aspekte“, W. Diebschlag, B. Diebschlag, (2000, Herbert Utz Verlag, München) if not other cited.

Mites belong to the group arachnida like spiders and ticks. Up to now, more than 30.000 different kinds of mites are known.

Associated with allergies, especially those mite species are relevant, which are found in greater amounts in the living area of the people. Approximately 50 different species are known so far in domestic life and in the storage room. The proportion of house dust mites of the total mite population in the human living area, especially in house dust, is about 80%. The most important species found in house dust and therefore correlated to allergic diseases are *Dermatophagoides pteronyssinus,* which is known since 1952, *Dermatophgoides farinae* and *Euroglyphus maynei.*

Figure 1: Mites eating a skin flake.[5]

The major food sources of dust mites are human dander and microorganisms, especially fungi. The fungi are used for pre-digestion of fatty skin flakes. 0.25 g of dander is sufficient to feed several thousand mites over several months. Mites seem to be omnivores as well as food products, pollen, bacteria and plant fibres were found in her gastrointestinal tract. The

consumption of dead conspecifics is also possible, since body parts of other mites were found in the digestive tract.

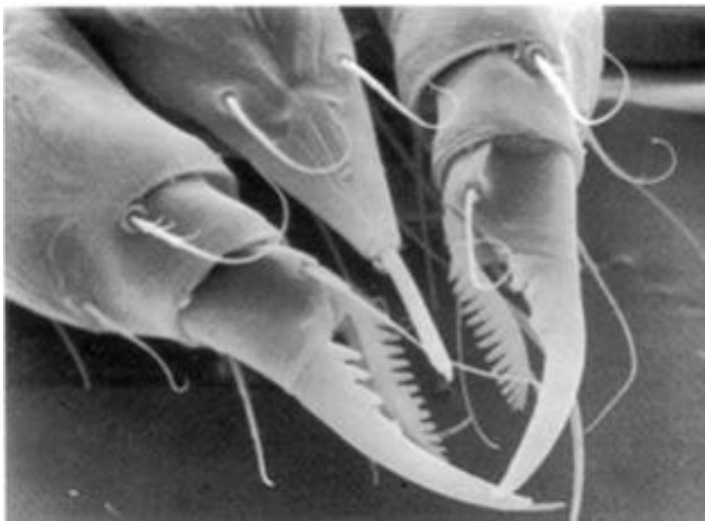

Figure 2: Mouthpart of a mite[5]

Dust mites are not visible by the naked eye due to their size of 0.1-0.5 mm. In most cases male mites are smaller than female ones. The body mass of mites consists of 70-80% water. The standard weight of *Dermatophagoides pteronyssinus* is with 5.83 ± 0.16 µg specified for females and 3.48 ± 0.16 µg for males. Their dry weight is 1.53 ± 0.99 µg and 0.96 ± 0.09 µg, respectively.

In the active state mites lose up to 3.4% of their total water content per day. They need to replenish the water content from outside, in order not to dry out. Most of the water is lost by egg production, the evaporation of water at the surface and the elimination of secretions and excretions. The water uptake is mainly via active and passive absorption of water from the air.

The optimum moisture conditions for dust mites are at 70-80% relative indoor humidity at common room temperatures. Putting dust mites 6-11 days at a relative humidity below 50% will kill all of them because the relative humidity is strongly correlated to their adsorption of water from the air.

Dust mites are poikilothermic, which means that they assume the same temperature as their surroundings. The optimal body temperature of the mites lies between 25-28 ° C. At higher temperatures their activity increases but their lifetime decreases. They can survive temperatures of 45 ° C for up to 24 hours. Reproduction is possible in the entire temperature

range of 10-37 ° C. If the temperature is below 10°C the mites are inactive. At a temperature of -1 ° C house dust mites are able to survive for 3 weeks.

Beds provide favourable condition for mites. The bed temperature during night is about 32-36 ° C. Heavy sweating can lead to higher humidity exceeding values of 50-70% and if a person takes a shower before going to bed the relative humidity can be risen up by 20% for a short time.

Investigations of van Bronswijk in the UK on mite population in different areas of apartments showed the following:

Table 1: Number of mites in different parts of the houses in the UK[6]

sources of dust	**house dust mites/g dust**
bed dust	4241
bedroom floor dust	1088
living room floor dust	274
floor dust from stairs	395
hallway floor dust	280
kitchen floor dust	80

These results demonstrate that in beds and bedrooms most dust mites occur. Other rooms are much less stressed. This can be due to the fact that other rooms are more cleaned and vacuumed and that there is a more unfavourable climate for mites. It has to be considered that in older houses higher dust mite allergen levels have been reported.[7] It is concerning that the number of living mites in children's rooms is really huge compared to other bedrooms. It is confirmed that at high altitudes the number of mites in house dust is lower.[8]

Table 2: Dust quantity, number of live and dead dust mites (HDM), and guanine content given by the Acarex-test of house dust samples from a household with a sensitized person against house dust mites.[9]

Room	**Object (per 0.25 m²)**	**Dust [g]**	**HDM alive**	**HDM dead**	**Acarex-test**
living room	furniture	59.0	0	13275	1-2
	pillow	14.8	0	3404	1
	carpet	44.0	0	784	0-1
children's room	sofa bed	39.0	3315	4017	2-3
	carpet	21.0	63	567	1
bedroom	bed	58.0	174	9454	1-2
kitchen	corner seat	60.0	0	2400	0-1

Mites have the ability to move around independently. They move slowly but persistently. Mites have so-called adhesive roots that allow them to cling to various materials. So they cannot be sucked up by the air suction of the vacuum cleaner. Over greater distances, the mites are spread essentially passive. People and animals move the mites in clothing, shoes, on the skin and the hair.

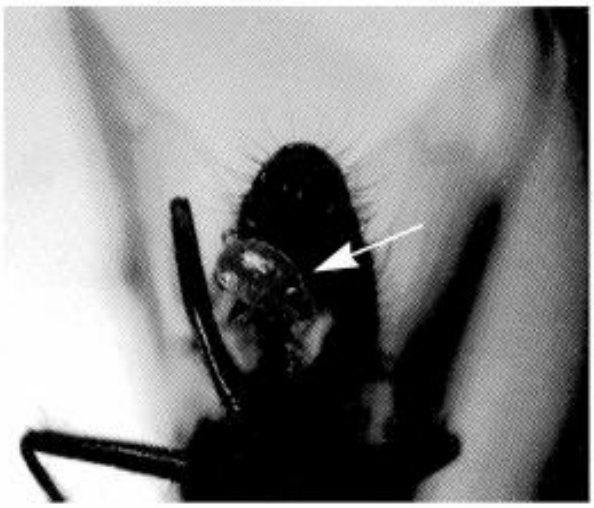

Figure 3: Mite holds on tight to fly and can be transported over long distances.[5]

Studies on the relationship between occurrence of house dust mites and animal husbandry yielded the following results: Dust samples from households with a cat were considered to be less contaminated with mites than samples from households without a cat. In contrast, they found higher concentrations of mites in dust samples from households with a dog or with a dog and cat.

2.2 Detection of house dust mites

2.2.1 Taking samples

2.2.1.1 Dust samples

With the vacuum cleaner dust samples can be taken. The method is simple, rapid and reproducible. A general problem with sampling is that house dust is not homogeneously distributed and dust mites can occur more frequently in some places. The amount of collected dead mites is between 55-90% while the amount of living mites is only 20%. This is due to their hooked legs with which they can cling to a substrate on which they reside.

2.2.1.2 Mobility method

A textile is coated with adhesive protective film. After 24 hours, the film is removed and the mites stick on it. Up to 30% of living dust mites of this study area can be detected.

2.2.1.3. Heat Escape Method

This method is based on the fact that at high temperatures mites migrate to cooler places. Again a sticky film is used to catch the mites, which is placed at the cooler location. A similar technique for the determination of the number of mites is the Berlese-Tüllgren extraction. A light bulb is heating the samples and the mites start to migrate. Instead of a sticky film the mites fall into a chemical solution which is deadly for them. Under the microscope the number of mites is counted which is simplified due to the fact that the mites are dead and cannot move anymore.[10]

As in the samples of the Mobility Method also only living mites are detected in the samples of the heat escape method. Generally the mites are counted under the microscope.

2.2.2 Detection

2.2.2.1 Counting mites under the microscope

By counting a certain amount of mites in the sample conclusions can be drawn about the entire population of mites. It is possible to distinguish between the different species, living and dead mites and the stages of development.

2.2.2.2 Immunochemical Assays

Immunochemical assays determine the amount of mite allergen by the use of cloned antibodies. Methods like RAST (radioallergosorbent test) and ELISA (enzyme-linked immunosorbent assay) are applied in the laboratory. The informational value and reliability of these methods are very high.

2.2.2.3 Guanine Colour Test (Acarex-test)

The Guanine Colour test determines the guanine concentration in house dust due to the fact that guanine is considered to be an indirect indicator for the contamination of mites.
(see chapter 3).

2.3 House dust mite allergy

In 1964, the house dust mite was identified as a trigger of house dust allergy. Moreover, house dust is one of the most important sources of inhalant allergens. House dust allergens can be found both in the dust and particles in the room air. The amount of allergens is approximately proportional to the number of mites. The most studied allergens in consideration of house dust are *Der p1* and *Der f1* (Der = *Dermatophagoides*, p = *pteronyssinus*, f = *farinae*). 1 refers to the group to which this allergen belongs. Group 1 allergens have compared to group 2 allergens a tenfold higher allergenicity. Group 1 allergens are thermally unstable, very soluble and stable in a pH range of 4-10. In contrast to group 2 allergens which are thermally more stable, soluble and stable in a pH range of 2-12.

Main diseases associated to house dust mite allergy are rhinitis (45%), asthma (34%) and atopic dermatitis (7%). Children are particularly affected by the dust mite allergy. Children 0-18 years with allergic diseases show 80% sensitization to house dust mites. From the 5 to

18 years old even 88% show positive reactions to dust mites whereas only 68% are sensitized to grass pollen. Studies investigated the correlation between the severity of atopic dermatitis in children and the indoor level of house dust mite allergens.[11]

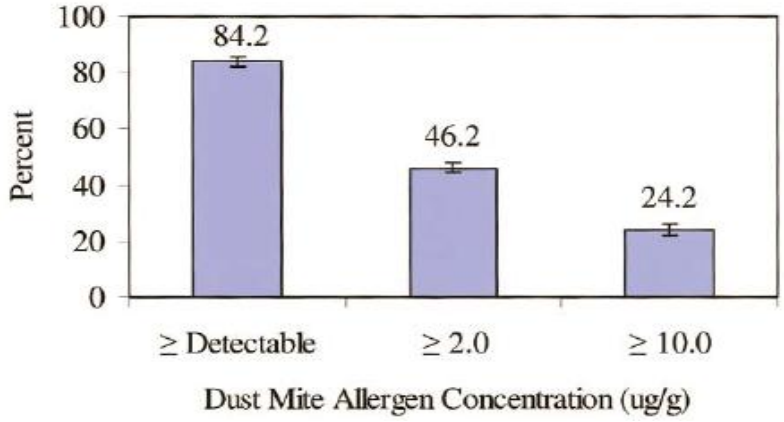

Figure 4: Percentage of US homes with dust mite allergens *Der f1* plus *Der p1*.[7]

In a study of US homes the majority of them (84.2%) had detectable levels of dust mite allergens in beds. Approximately one half of the homes had levels of greater than 2 μg/g, the proposed threshold for allergic sensitization, and approximately one quarter had levels at or greater 10 μg/g, the proposed threshold for asthma.[7]

2.4 Avoidance of house dust mites

First of all it should be mentioned that mattresses which are older than 8 to 10 years should be replaced by new ones. The encasing of mattresses (e.g. using polyurethane encasings) has been proven to be a successful method for the reduction of house dust mite allergen levels.[12, 13, 14] Weekly change and wash of bed linen at a temperature between 60 °C and 95 °C also reduce a significant number of mites (see Table 3).

Table 3: Reduction of living mites after washing at different temperatures[15]

conditions	reduction of house dust mites in %
Washing at 30°C	97.0
Washing at 60°C	99.3
Washing at 90°C	100

It was also proven that children which uses feather quilts were less likely sensitized to house dust mites and therefore less likely to have severe asthma symptoms. *Der p* allergen levels are significantly lower in feather pillows.[16]

Not only the reduction of house dust mites in the bed are of importance but also the reduction of the mites in the whole living areas of humans. It has been demonstrated that the lowering of indoor relative humidity through the use of a high-efficiency dehumidifier and air conditioning can result in significant reductions in dust mite numbers and allergens.[17] As the relative indoor humidity plays in important role the heating of bedrooms should not be omitted in the winter because the heat lowers the relative humidity, which leads to a strong reduction of dust mites at least in winter.

It was demonstrated that exposing carpets to sunlight for 6 hours created a microenvironment lethal to mites as well as that air out the bedding of the window and this way of exposure to light may has the same effect.[18]

Other avoidance methods include the removal of carpet, vacuuming with the consideration that only a part of house dust mites is killed and regularly venting of living areas. Further stuffed toys are a common living environment of mites so toys should be put into the freezer or washed at minimum 60 °C for 60-90 min twice a month.

3 Determination of guanine

The guanine concentration in house dust samples is an indirect indicator for the amount of allergenic mite faecal pellets due to the fact that guanine is the major nitrogenous waste product of arachnids.[19] The guanine levels in homes where *Dermatophagoides pteronyssinus* (*Der p*) patients experienced symptoms were significantly higher than in homes where these patients were symptom free. Good correlation between the mite allergenicity of different house dust samples and their guanine content were determined. Further, it was investigated that people allergic to house dust but not to *Der p* showed no allergic reactions in presence of guanine-rich house dust.[19]

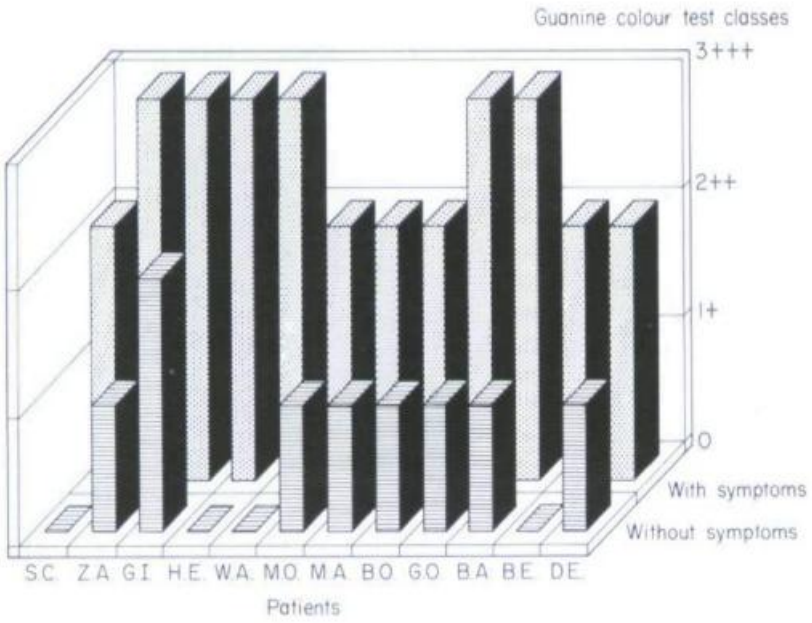

Figure 5: Guanine levels in house dust samples (given by the Acarex-test) from homes where people allergic to *Der p* showed allergic symptoms (background), and from homes where these people were symptom free (foreground).[19]

3.1 Acarex-test

For semi-quantitative prediction of mite allergens the Acarex-test is used in many households because it can be conducted easily and does not need any chemical equipment. The determination of guanine is enabled by a visible chemical reaction to a guanine azo-dye. The Acarex-test is based on the correlation between the colour intensity and the guanine concentration.[20] After extraction of the dust in an alkaline solution composed of potassium

hydroxide, methanol and water the test is evaluated by visual comparison of a colour stick with a colour table. The Acarex-test is used both scientifically[21,22] and domestically. The World Health Organisation set the borderline level of guanine to be 0.6 mg/g of dust.[20]

Table 4: Acarex-test and corresponding guanine ranges.[20]

Acarex step	0	1	2	3
guanine range	0.0-0.6 mg/g	0.6-2.5 mg/g	2.5-10.0 mg/g	> 10 mg/g

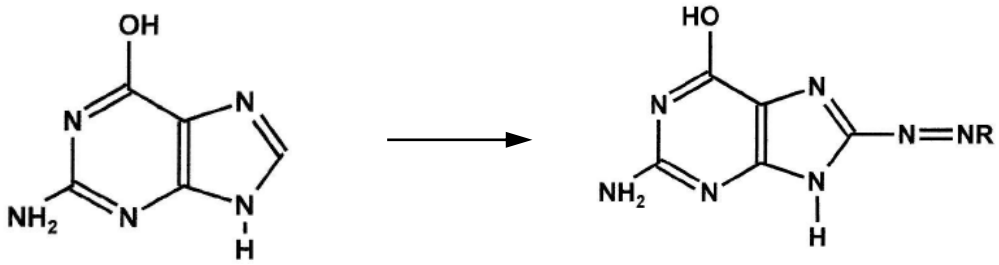

Figure 6: Comparison of structures of guanine and guanine azo dye [20]

As mentioned before the mite allergen levels which are considered as risk factors for sensitization are 2 μg/g and 10 μg/g of *Der p 1* and *Der f 1*. It was investigated that house dust samples with class 2 and 3 definitely contained more than 2 μg/g of *Der p 1* and *Der f 1*. The probability that household dust which is classified as class 2 or 3 in the Acarex-test contained more than 10 μg/g of mite allergens is 88% and 100%, respectively.[23]

3.2 Determination of guanine with HPLC

E.R.C. Bischoff et. al. performed quantitative guanine determination by HPLC. The guanine was extracted from the dust samples by an alkaline mixture. In HPLC guanine is detected at a wavelength of 254nm.[20] In this thesis guanine extraction was performed using an acid as extraction solvent as guanine is soluble in alkaline as well as acidic solutions.[24, 25]

3.3 Determination of guanine with GC/MS

GC/MS measurements of guanine are more time-demanding compared to HPLC measurements because the guanine has to be derivatized after extraction. Generally, derivatization modifies the chemical structures and enables better analysis of compounds. Three types of derivatization are widely used, alkylation, acylation and silylation which is preferred in GC measurements. Silylation displaces active protons in –OH, -COOH, =NH, $-NH_2$ and –SH groups by the trimethylsilyl group [$Si(CH_3)_3$]. Silylation is necessary because otherwise the active hydrogens form intermolecular hydrogen bonds which affect the inherent volatility, the interactions with column packing materials and the thermal stability. Silylation results in reduced polarity, enhanced volatility and increased thermal stability. The disadvantage of silylation reagents and silylated derivatives is that they are hydrolytically unstable which means that they have to be protected from moisture.[26] The general reaction scheme is shown in Figure 7.[27]

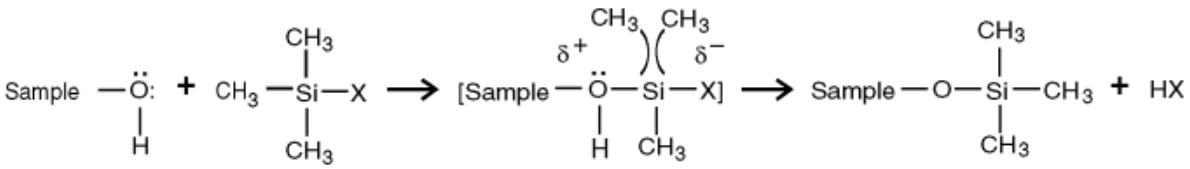

Figure 7: General reaction mechanism of trimethyl silylation[27]

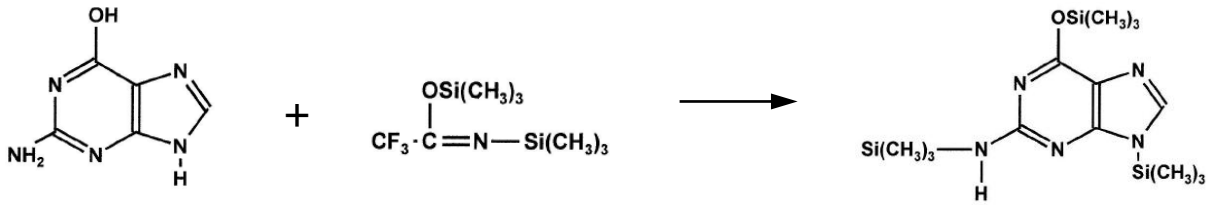

Figure 8: Reaction mechanism of guanine with BSTFA

4. Ergosterol

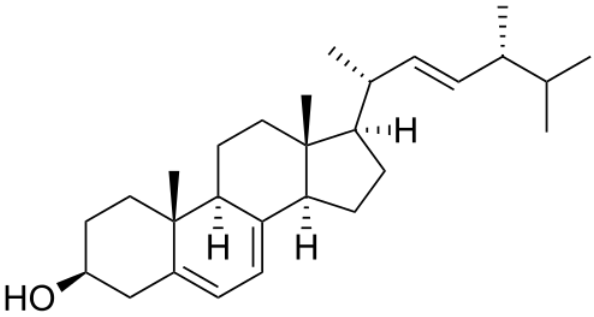

***Figure 9*:** Structure of ergosterol ((22E)-Ergosta-5,7,22-trien-3β-ol)

Ergosterol plays an important role for scientific purpose. It is the major sterol constituent of most fungi[28] and therefore serves as a biomarker for the presence of fungal contamination since the late 1970s.[29] It has been used as an indicator for determining fungal biomass in soil, feedstuff, sediments, organic dust, settled house dust, indoor aerosols and bio contaminated building materials[30], decaying plant materials and aquatic systems.[31] Other possible compounds strongly correlated with fungal contamination are chitin[28] and ATP. ATP is not an appropriate biomarker as it can be obtained from many different sources.[29] The main advantage of ergosterol is its specific association with fungi as it is absent in most higher plants. Another considerably fact is that ergosterol only indicates live fungal mass because ergosterol is labile and becomes rapidly oxidised upon cell death.[28] Ergosterol is also degraded by UV radiation converting it into ergocalciferol (Vitamin D_2).[29] It was reported that low pH is a reason for instability of ergosterol.[32]

The amount of ergosterol in fungal tissue is not constant and varies between 0.4 and 42 μg/mg dry weight and is highest in yeast. The content of ergosterol is influenced by fungal species, humidity, age of culture, developmental stage and growth phase of the fungi.[31]

In the daily life humans are persistently exposed to fungi without appearing of any harmful health effects to the human body but is has to be considered that fungal cells and spores are potential health hazards.[30] Illnesses caused by fungi and moulds include hypersensitivity pneumonitis[33], asthma, rhinitis, bronchitis[34], cough and wheeze. Also unspecific symptoms like tiredness and headaches are caused by dampness in buildings.[35] Mould allergies are responsible for 25-30% of all allergic asthma cases.[30] The majority of fungi and mould

spores inhalated by humans are from sources within buildings because people spend 85-90% of their time indoors. However, mould growth will only occur at a relative humidity greater than 60% and sufficient warmth to allow mould spore germination and growth.[34]

An astonishing fact is that also cigarette smoke contains fungal components. Szponar et al. reported that the air concentration of ergosterol is significantly higher in rooms with ongoing smoking than in rooms without smoking. The fungi grow naturally on the tobacco plant and survive the combustion during smoking. The average ergosterol content in rooms without ongoing smoking is 1.2 ng/m^3 and rises to 18.2 ng/m^3 while smooking.[36]

5 EXPERIMENTAL

5.1 Sample collection

Dust samples were collected from six households and a nursery school. Fine dust particles were taken out of the vacuum cleaner bags or form the dust sampling container of the vacuum cleaners. In this study five samples were collected from vacuum cleaner bags and two from sampling containers.

5.2 Determination of guanine by liquid chromatography

5.2.1 Materials and reagents

All chemicals were used as received. Acetonitrile (Optima LC/MS Grade) was purchased from Fisher Chemical. Guanines (puriss 99%) as well as ammonium acetate were obtained from Fluka. Hydrochloric acid (35%) was purchased from Lachner.

5.2.2 Sample Preparation

Guanine was extracted using solid-liquid extraction. The extraction solvent used was 0.1M HCl. For extraction 0.2 g dust were overlaid with 2 mL of solvent. The suspensions were vortexed and shaken for 2 hours in the dark. Then the solutions were let stand in the dark for another 22 hours. After 1 day of extraction the samples were vortexed and put into ultrasonic bath for 30 minutes. Afterwards, the crude particles were filtered off and a clear solution containing guanine resulted. All samples were filled up with 0.1 M HCl to the same volume of 1.4 mL.

5.2.3 Preparation of standard solutions

Identification of the product in LC was done by using external standards with different concentration of guanine in 0.1 M HCl. A solution of 1 mg/mL of guanine in 0.1 M HCl was diluted to concentrations of 25 μg/mL, 12.5 μg/mL, 5 μg/mL, 2.5 μg/mL and 0.5 μg/mL.

5.2.4 Instrumentation

Products from the solid-liquid extraction were analysed by injection of liquid samples into LC. Quantitative analyses were performed on an LCQ Accela Fleet (Thermo Fisher Scientific, San Jose, CA, USA) equipped with an autosampler, an atmospheric pressure chemical ionization (APCI) source and a photodiode array detector. APCI capillary temperature was 275 °C, APCI vaporizer temperature 300 °C, sheath gas flow 58 L min^{-1}, auxiliary gas flow 10 L min^{-1}, source voltage 6 kV, source current 5 μA, and capillary voltage 22 V. A ZIC-HILIC, 150 x 2.1 mm, 3.5 ìm, 200Å (Merck) column was used. Acetonitrile/NH_4Ac 5mM 80/20 (v/v) was used as mobile phase and the flow rate was set at 250 μL/min. The UV detection wavelength was set at 250 nm for acquiring chromatograms and quantitative analysis. The MS spectra were acquired in positive ion mode.

5.3 Determination of guanine by gas chromatography

5.3.1 Materials and reagents

All chemicals were used as received. Guanine (puriss 99%) was obtained from Fluka. Nonadecanoic acid and hexane were purchased from Larodan and Merck respectively. Pyridine and BSTFA were both obtained from Sigma-Aldrich.

5.3.2 Sample Preparation

From each extract prepared for LC analysis 200 μL were transferred into 2 mL vials which were covered with aluminium foil with tiny holes in it. The vials were put into the deep freezer at -80°C for a short time. Afterwards the samples were put into the lyophilizer overnight.

To the residues 25 μL of internal standard (nonadecanoic acid, concentration 100 μg/mL in hexane) were added and as well evaporated under stream of nitrogen.

Derivatization: The residues were dissolved in 80 μL of pyridine + 60 μL of BSTFA (1% TMCS). The samples were vortexed well and heated at 60°C for 70 minutes. Afterwards pyridine and BSTFA were evaporated under stream of nitrogen and the residues were immediately redissolved in 100 μL of hexane/BSTFA (5%) mixture.

5.3.3 Preparation of standard solutions

Identification of the product in GC/MS was done by using external standards with different concentration of guanine as well as an internal standard. A solution of 1 mg/mL of guanine in 0.1M HCl was diluted to concentrations of 100 μg/mL, 50 μg/mL, 25 μg/mL, 10 μg/mL, 5 μg/mL and 2 μg/mL.

250 μL of each standard were transferred into a 2 mL vials, covered with aluminium foil into which tiny holes were made. The vials were put into the deep freezer at -80°C for a short time. Afterwards the samples were put into the lyophilizer overnight. To the residues 62 μL of internal standard (nonadecanoic acid, concentration 100 μg/mL in hexane) were added and as well evaporated under stream of nitrogen.

Derivatization: The residues were dissolved in 80 μL of pyridine + 60 μL of BSTFA (1% TMCS). The samples were vortexed well and heated at 60°C for 70 minutes. Afterwards pyridine and BSTFA were evaporated under stream of nitrogen and the residues were immediately redissolved in 250 μL of hexane/BSTFA (5%) mixture.

5.3.4 Instrumentation

The gas chromatograph was equipped with a fused silica column (Restek, ZB-5-MS). Injections were made in the splitless mode; helium at a flow rate of 1 mL/min was used as carrier gas. The injector was kept at 250°C and the transfer line at 250°C.

Temp. program: 120°C/1 min → 15°C/min → 210°C/0min → 5°C/min → 260°C/0 min

Post Run conditions: 305°C/5min

The chromatograms were evaluated using different MS setups for different retention times. 1st segment: Full Scan – 50-600 AMU (5 - 9 min); 2nd segment: MS2, precursor ion m/z 352 (9 - 11:30 min); 3rd segment: Full Scan – 50-650 AMU (11:30 – 17 min);

5.4 Determintation of guanine by Acarex-test

5.4.1 Materials and reagents

All chemicals were used as received. Guanine (puriss 99%) was obtained from Fluka. KOH as well as MeOH were purchased from Lachema.

5.4.2 Sample Preparation

Usually the Acarex-test is performed in alkaline solution. The extraction solution was prepared on our own in accordance with the instructions of use of the test. For that 1.33 g KOH pellets were dissolved in 8.8 mL H_2O and 33.1 mL MeOH. Approximately 130 mg of dust were weighed of each sample which corresponds to 1 spoon of dust and diluted with 1.2 mL of extraction solvent. The sample was shortly vortexed and the test strip was dipped into the solution. After 1 minute it was compared to the colour scale.

5.4.3 Preparation of standard solutions

For checking if the Acarex test works properly standard solutions were prepared for each class of contamination. For class 0, 1, 2, 3 guanine concentrations of 0.3 mg, 1.4 mg, 5.9 mg and 12.4 mg in 2 mL of extraction solvent were prepared, respectively. For testing also a blank was used.

5.4.4 Performing Acarex-test with extracts used for LC and GC/MS measurements

As mentioned above the Acarex-test is performed with an alkaline extraction solvent. The extracts used for LC and GC/MS measurement were in 0.1 M HCl. Therefore twice 250 µL of Oma and Dejan extracts were transferred into 2 mL vials, respectively. The duplicates of the samples were then deep frozen and lyophilizated. After lyophilization the samples were dissolved in 250 µL of the extraction solvent used for Acarex test and a test strip was dipped into the mixture.

5.5. Determination of ergosterol

5.5.1 Materials and reagents

All chemicals were used as received. Guanine (puriss 99%) was obtained from Fluka. NaOH, cholecalciferol (puriss 99%), pyridine and BSTFA were purchased from Sigma-Aldrich. Hydrochloric acid (35%) was purchased from Lachner. Hexane and MeOH were obtained from Merck and Lachema respectively.

5.5.2 Sample Preparation

Approximately 500 mg of dust were weighed into 22 mL vials. Then 6 mL MeOH and 2 mL of 2M NaOH were added. The samples were heated in the microwave oven at 600 W two

times for 25 seconds. The samples were cooled down by water after each heating step. The contents were neutralized by 3 mL of 1M HCl (tested by pH paper). Additional 3 mL of MeOH were added. Afterwards the ergosterol was extracted three times by 4 mL hexane (addition of 4 mL hexane, 10 min shaking, centrifugation at 2000 rpm for 4 minutes, hexane phase transferred to new vial). The extracts were joined and evaporated under stream of nitrogen, then dissolved in 1 mL hexane and derivatized.

Derivatization: Into 2 mL vials 15 µL of ISTD (cholecalciferol 1 mg/mL in toluene) were transferred and then diluted with 250 µL of the sample. This mixture was then evaporated under stream of nitrogen. The residues were dissolved in 50 µL of pyridine and 70 µL of BSTFA (1% TMCS) and heated at 60°C for 30 minutes. Afterwards pyridine and BSTFA were evaporated under stream of nitrogen and the residues were dissolved in 750 µL hexane.

5.5.3 Preparation of standard solutions

Identification of the product in GC/MS was done by using external standards with different concentration of guanine as well as an internal standard. A solution of 1 mg/mL of ergosterol in hexane/toluene (1:1) was diluted with hexane to concentrations of 100 µg/mL, 50 µg/mL, 25 µg/mL, 10 µg/mL, 5 µg/mL and 2 µg/mL. 250 µL of each standard solution were transferred into new vials and evaporated under nitrogen. Afterwards 10 µL of ISTD (cholecalciferol, 1 mg/mL in toluene) were added and also evaporated under nitrogen.

Derivatization: The residues were dissolved in 50 µL of pyridine and 70 µL of BSTFA (1% TMCS) and heated at 60°C for 30 minutes. Afterwards pyridine and BSTFA were evaporated under stream of nitrogen and the residues were dissolved in 500 µL hexane.

5.5.4 Instrumentation

The gas chromatograph was equipped with a fused silica column (Restek, ZB-5-MS). Injections were made in the splitless mode; helium at a flow rate of 1 mL/min was used as carrier gas. The injector was kept at 280°C and the transfer line at 250°C.

Temperature program: 100°C/0,5 min → 35°C/min → 260°C/0min → 2,5°C/min → 308°C/5min

The chromatograms were evaluated by MS full scan 50-600 AMU.

6 RESULTS AND DISCUSSION

6.1 Determination of guanine by liquid chromatography

6.1.1 Determination of suitable extraction solvent

First of all, the extraction parameters had to be defined, including the extraction solvent and the time of extraction. For these experiments household dust from Oma was taken. Therefore different extraction solvents like 0.1M HCl, water and the mobile phase were tested. The mobile phase used consisted of 80% acetonitrile and 20% 5mM CH_3COONH_4. The best extraction solvent was 0.1M HCl as the yield of guanine was highest. The amounts of guanine obtained from all three extraction solvents are shown in Table 5. Moreover, these results confirm that guanine is almost insoluble in water.

Table 5: Amounts of guanine obtained with different extraction solvents

extraction solvent	**guanine [µg/g dust]**
0.1M HCl	42.611
H_2O	19.598
mobile phase	24.866

6.1.2 Determination of extraction time

After determination of the extraction solvent the extraction time was defined using the best extraction solvent - 0.1M HCl. About 0.2 g of dust were mixed with 2 mL of extraction solvent. First, the extraction after 30 minutes in the ultrasonic bath was tested. The samples of the 3 hours and 24 hours extraction were shaken for 2 hours, then let stand in the dark for an additional hour or 22 hours, respectively. After total extraction time of 3 or 24 hours the samples were put into the ultrasonic bath for 30 minutes. According to these results, which are figured out in Table 6 the most sufficient extraction time for guanine is 24 hours.

Table 6: Amounts of guanine obtained at different extraction times

extraction time	guanine [μg/g dust]
30 min ultrasound	42.623
3 hours extraction	43.553
24 hours extraction	60.771

6.1.3 Calibration – LC/UV detection

An equation for the calibration curve of the target compound was obtained as Y=98296X-10051, where Y and X were the value of peak area and the concentration of standard solution (μg/mL), respectively. Linear regression of the standard showed good linearity with a correlation coefficient (R^2) of 1 as it can be seen in Figure 10. The contents of guanine in the test samples were then calculated using the established regression equation. For the conditions of the measurements see Experimental part 5.2.4.

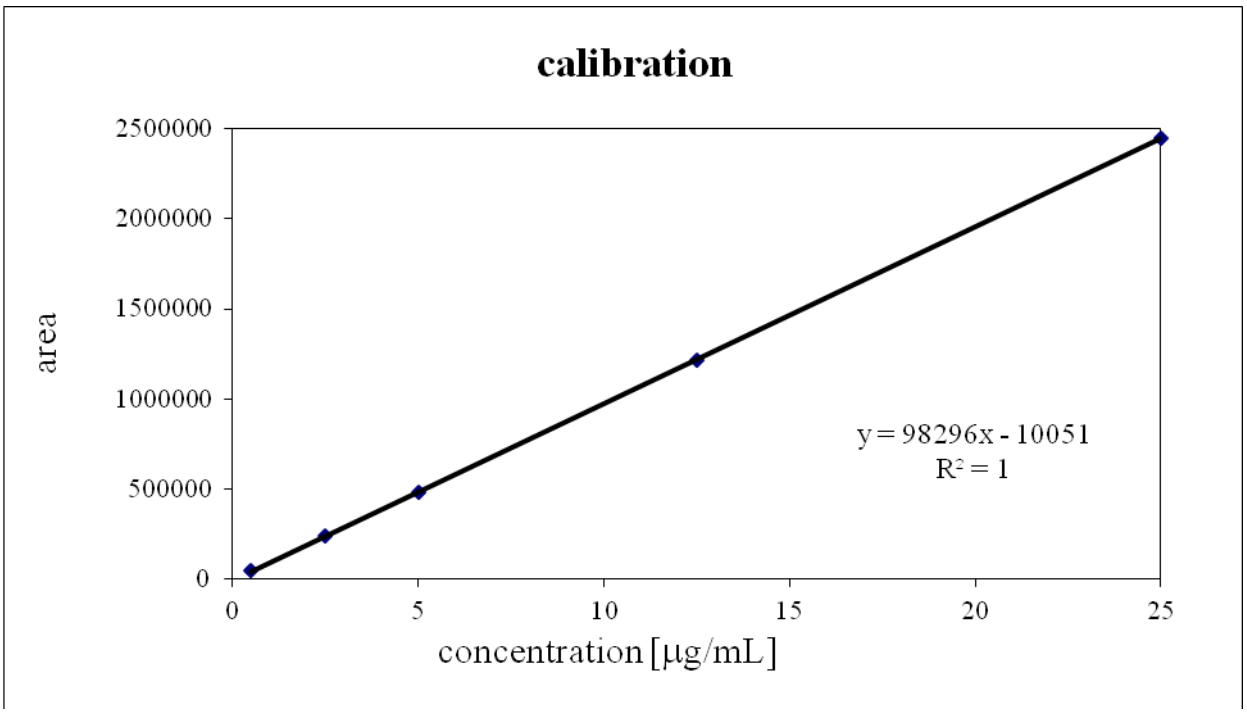

Figure 10: Calibration line of guanine for LC/UV measurements

6.1.4 Concentration of guanine in different house dust samples – LC/UV detection

According to our measurements the amount of guanine in the house dust samples ranged from 4 – 48 µg/g of dust. The individual results are shown in Table 7 while Figure 11 shows a visible comparison of the guanine contents of the samples. The gained results are compared to the Acarex-test (see section 6.3). According to studies of Bischoff et al. guanine concentrations in house dust were determined between 0.02 – 21.7 mg/g of dust. The World Health Organisation set the borderline level of guanine to be 0.6 mg/g of dust.[20] In comparison to these literature values the amount of guanine in the tested house dust samples is quite low. (further discussion section 6.3)

Table 7: Results from LC/UV measurements

	amount of guanine [µg/g dust]	**standard deviation [µg]**
Dejan	4.011	0.172
Tanja	14.979	0.872
Maria	5.941	0.098
Ulrike	18.897	2.512
Oma	48.251	4.318
KiGa	12.262	0.491
Herta	N/A	N/A

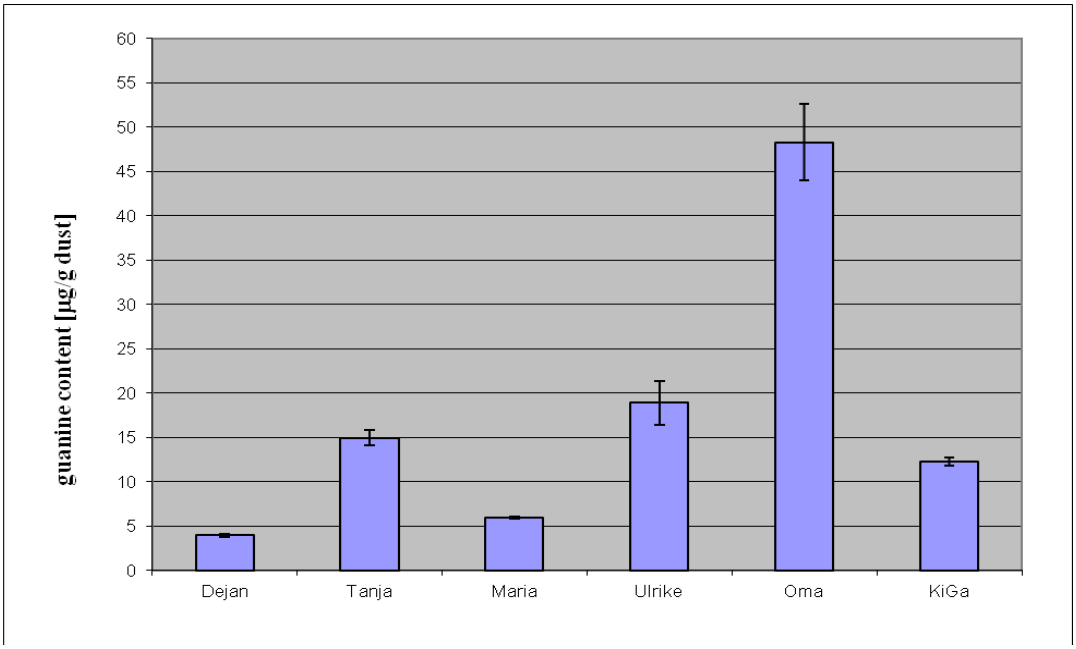

Figure 11: Differences in guanine concentration in the house dust samples

6.1.5 Calibration – LC/MS detection

An equation for the calibration curve of the target compound was obtained as Y=22029X-15290, where Y and X were the value of peak area of guanine (*m/z* 152) and the concentration of standard solution (μg/mL), respectively. Linear regression of the standard showed good linearity with a correlation coefficient (R^2) of 0.9953 as it can be seen in Figure 12. For the conditions of the measurements see Experimental part 5.2.4.

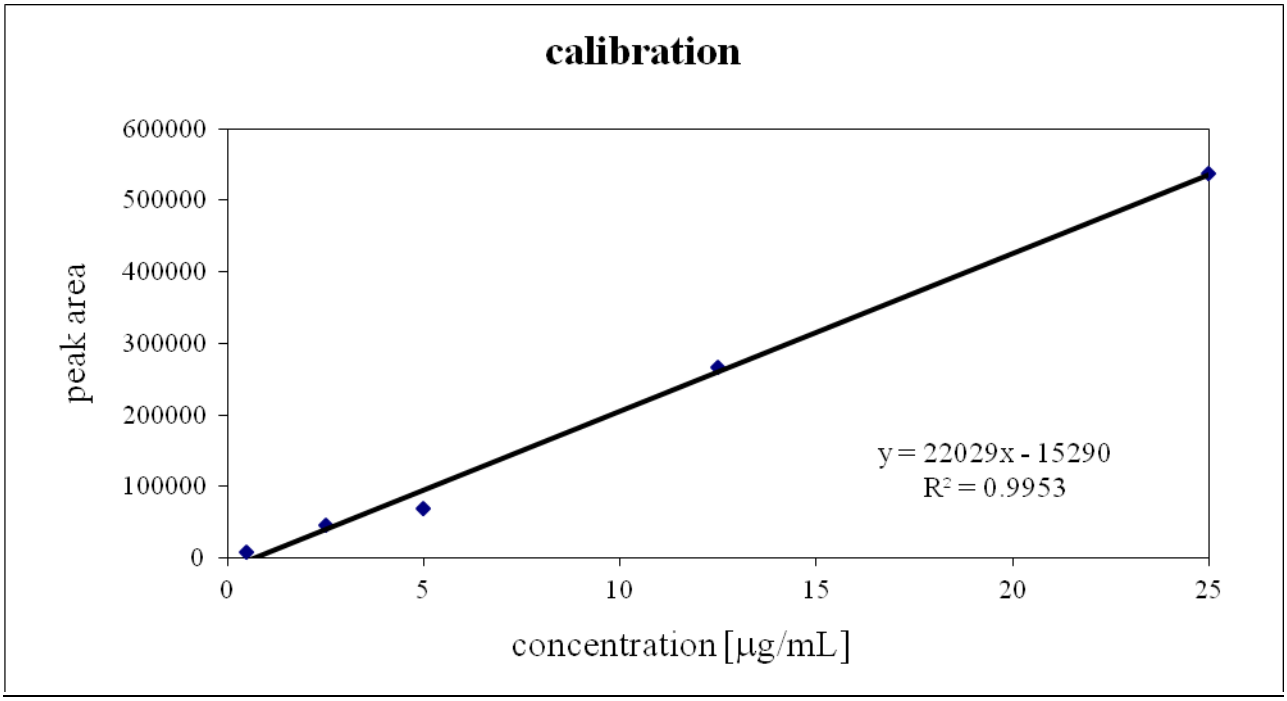

Figure 12: Calibration line of guanine for LC/MS measurements

6.1.6 Concentration of guanine in different house dust samples – LC/MS detection

In the LC/MS spectrum guanine can be detected but the amount of guanine cannot be quantified due to some overlay of the guanine peak with a peak of unknown substance. The unknown substance dominates the area of the peak. It can be concluded that guanine is present in low concentration in the different house dust samples.

6.2 Determination of guanine by gas chromatography - mass spectrometry

6.2.1 Calibration

An equation for the calibration curve of the target compound was obtained as Y=0.0086X-0.1156, where Y and X were the value of the ratio of the peak area of guanine (*m/z* 152) and the peak area of the internal standard (nonadecanoic acid), and the concentration of standard solution (μg/mL), respectively. Linear regression of the standard showed a linearity with a correlation coefficient (R^2) of 0.9854 as it can be seen in Figure 13. The contents of guanine in the test samples were then calculated using the established regression equation. For the conditions of the measurements see Experimental part 5.3.3.

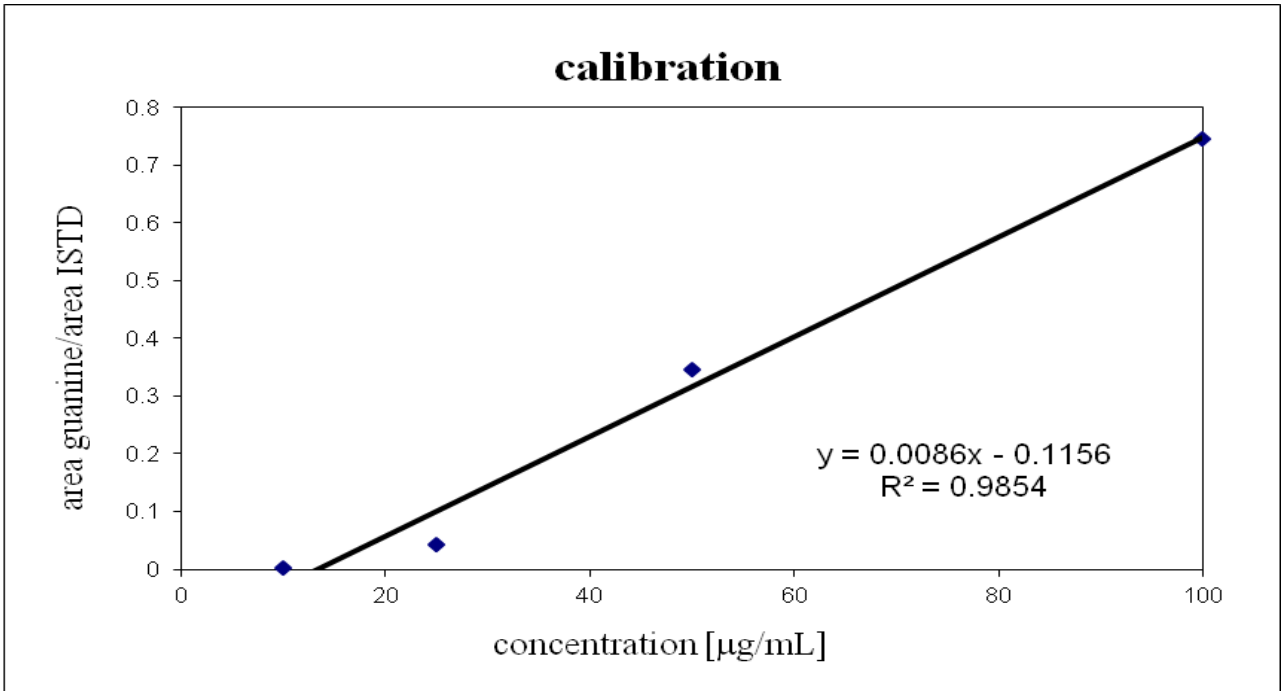

Figure 13: Calibration line of guanine for GC/MS measurements

6.2.2 Concentration of guanine in different house dust samples

The results obtained from GC/MS measurements (Table 8) are quite different compared to the results from LC/UV measurements (Table 7). Good agreement between LC/UV and GC/MS results was found in the case of Oma sample. Still relatively close are the results from Ulrike sample. On the one hand guanine was detected in the house dust of Herta by GC/MS measurement which was not the case in LC/UV measurements but on the other hand guanine was not detected in the samples of Maria and Kindergarten. The overall recovery should be tested including extraction and silylation of guanine. The reproducibility of the LC/UV results was much better than for GC/MS measurements, because the silylated derivative of guanine is not stable enough and further research is needed in order to establish very good and reproducible method.

Table 8: Results from GC/MS measurements

	amount of guanine [µg/g dust]	**standard deviation [µg]**
Dejan	14.769	1.057
Tanja	28.168	1.405
Maria	N/A	N/A
Ulrike	11.674	0.507
Oma	45.433	7.170
KiGa	N/A	N/A
Herta	10.835	0.446

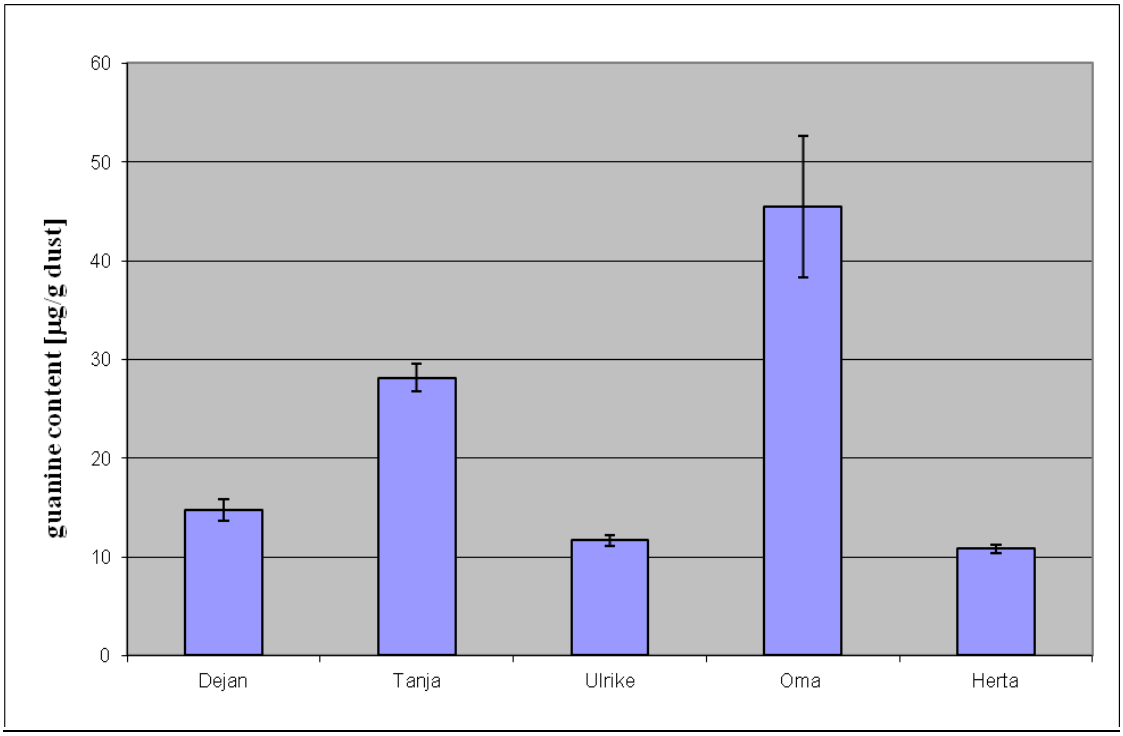

Figure 14: Results obtained for guanine concentration by GC/MS measurements

6.3 Determination of guanine by Acarex-test

The Acarex-test indicates the contamination by house dust mites in dust samples with a colour reaction. In Figure 15 the colour scale which resulted from the standard solutions can be seen. Also a blank was used which consisted of the extraction solvent only. It was used for the evaluation of the contamination of the samples. The calibration resulted in a nice colour gradient which indicates that the Acarex-test works properly in the specified ranges according to E.R.C. Bischoff et al..[20] For comparison Figure 16 shows the colour scale given by the instructions of use of the Acarex-test.

Figure 17 depicts the results gained from testing the samples. Most of the samples did not show any contamination and were therefore classified as class 0. The sample of Oma and Ulrike were classified as 1, and between 1 and 0, respectively. It can be concluded that the Acarex-test shows the same trend as the measurements by liquid chromatography and GC/MS measurements. According to the Acarex-test guanine concentrations must be much higher than gained from liquid chromatography measurements especially for Oma and Ulrike. One possible reason for the difference in the amount of guanine can be that usually the dust used for the Acarex-test is obtained separately from each textile object. In our case dust from the vacuum cleaner bags was used which means that the density of guanine is lower. Another explanation would be that the extraction efficiency was quite low. Extraction of guanine in alkaline solution was reported in some papers.[20] For our experiments the extraction of guanine was performed with 0.1M HCl as extraction solvent because of the LC instrumentation. Nevertheless, good extraction efficiencies of guanine with acidic extraction solvents must be achievable because guanine is soluble in slightly acidic solutions.

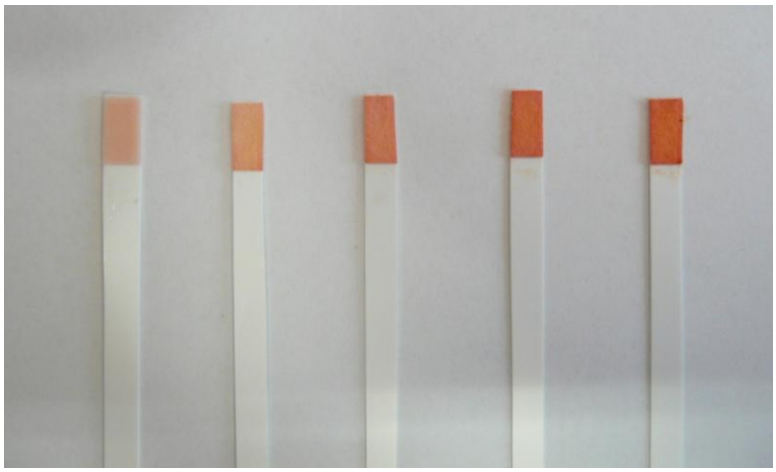

Figure 15: Comparison of colour intensities on the test stripes according blank, class 0, 1, 2, 3.

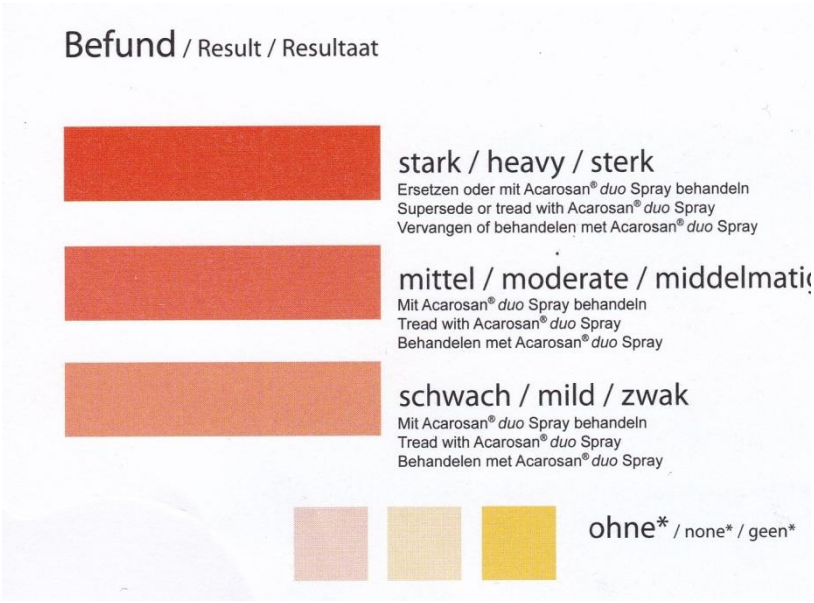

Figure 16: Colour scale given by the instructions of use of the Acarex-test

Figure 17: Comparison of samples with the colour scale of the standard solutions, blank, class 0, 1, 2 ,3, Tanja, Dejan, Oma, Maria, Ulrike, KiGa, Herta

6.4 Determination of ergosterol

6.4.1 Calibration

An equation for the calibration curve of the target compound was obtained as Y=0.0664X-0.0869, where Y and X were the value of the ratio of the peak area of ergosterol (*m/z* 363, 468) and the peak area of the internal standard (cholecalciferol), and the concentration of standard solution (μg/mL), respectively. Linear regression of the standard showed a good linearity with a correlation coefficient (R^2) of 0.9998 as it can be seen in Figure 18. The contents of ergosterol in the test samples were then calculated using the established regression equation. For the conditions of the measurements see Experimental part 5.5.4.

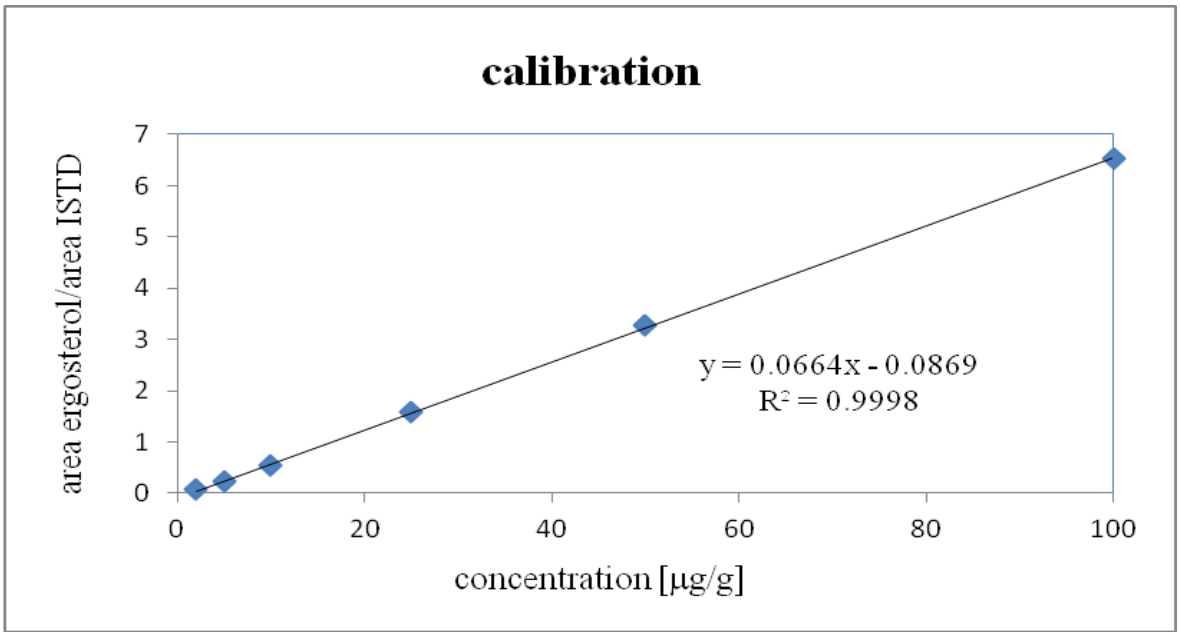

Figure 18: Calibration line of ergosterol for GC/MS measurements

6.4.2 Concentration of ergosterol in different house dust samples

Besides ergosterol also other sterols like cholesterol, β-sitosterol and campesterol were determined and quantified. Cholesterol is the most important sterol in animals as it is the major constituent of animal plasma membranes. In the membrane cholesterol regulates the fluidity but it is also the metabolic precursor to fat-soluble vitamins and steroid hormones. Plants and microorganisms contain instead of cholesterol structurally related sterols, they

differ only in their alkyl side chains. Sterols of plants are referred as phytosterols which include β-sitosterol and campesterol.[37]

Figure 19 outlines that the amount of cholesterol in most of the samples is much higher compared to the other sterols mentioned. Figure 20 depicts the differences in the concentrations of ergosterol, β-sitosterol and campesterol in the dust samples. The amount of ergosterol ranges from 8 - 9.8 μg/g of dust as it is depicted in Table 9. These values are close to the value reported by Parsi et al. who determined ergosterol concentrations of ~ 6 μg/g of household dust.[28] According to Axelsson et al. the quantity of ergosterol in house dust ranges from 6 - 45 μg/g of dust.[38]

The results show higher concentrations of β-sitosterol in the samples of Tanja and Oma compared to the other dust samples. It has to be mentioned that both households do not have a significant higher number of flowers and plants indoors. Both houses are surrounded by a garden but that is the case with almost all other households tested.

Table 9: Amounts of ergosterol, cholesterol, β-sitosterol and campesterol determined in the different house dust samples

	ergosterol [μg/g dust]	**cholesterol [μg/g dust]**	**β-sitosterol [μg/g dust]**	**campesterol [μg/g dust]**
Maria	11.388	52.744	46.836	19.492
Oma	19.811	2694.722	106.068	53.041
Dejan	8.274	3178.286	58.415	N/A
Ulrike	8.438	4987.004	92.152	42.164
Tanja	8.597	1919.582	144.341	57.887
Herta	11.242	946.776	81.401	27.033
KiGa	11.086	311.121	44.272	17.188

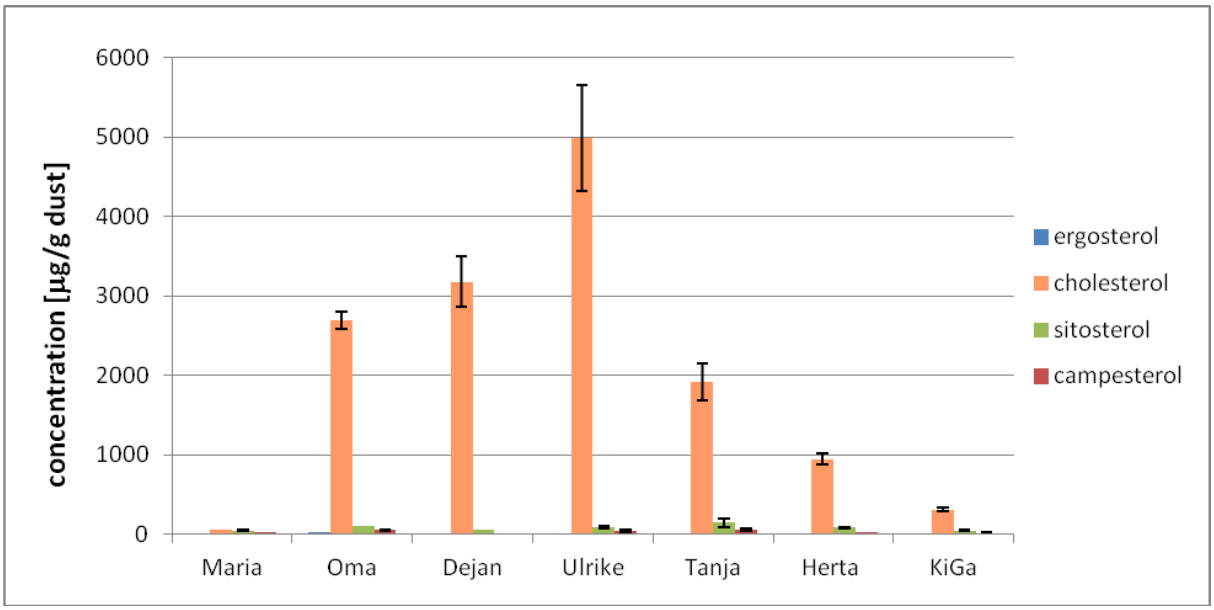

Figure 19: Comparison of the concentrations of sterols in the different dust samples

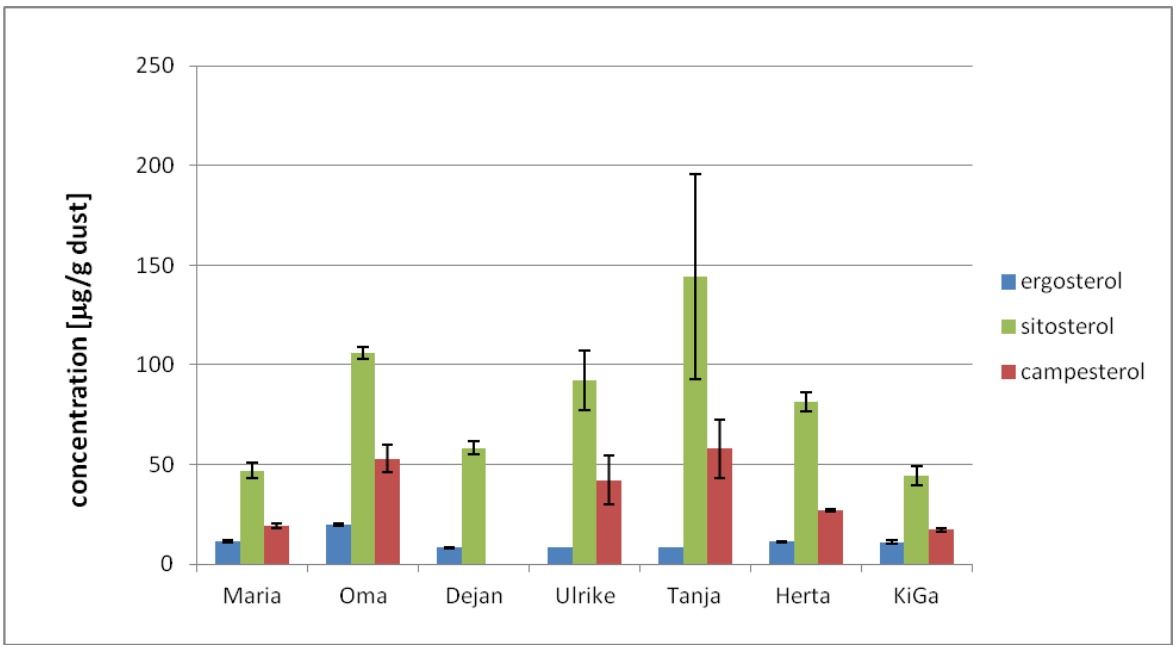

Figure 20: Comparison of the amounts of ergosterol, β-sitosterol and campesterol in the different dust samples.

Figure 21 shows the amount of guanine and ergosterol in the different house dust samples. It is conspicuous that the concentration of both guanine and ergosterol are higher in the sample of Oma. There is a connection between house dust mites and fungal growth in domestic homes due to the fact that both prefer a warm and humid environment. This assumption cannot be verified with the achieved results because the number of different house dust samples was too low.

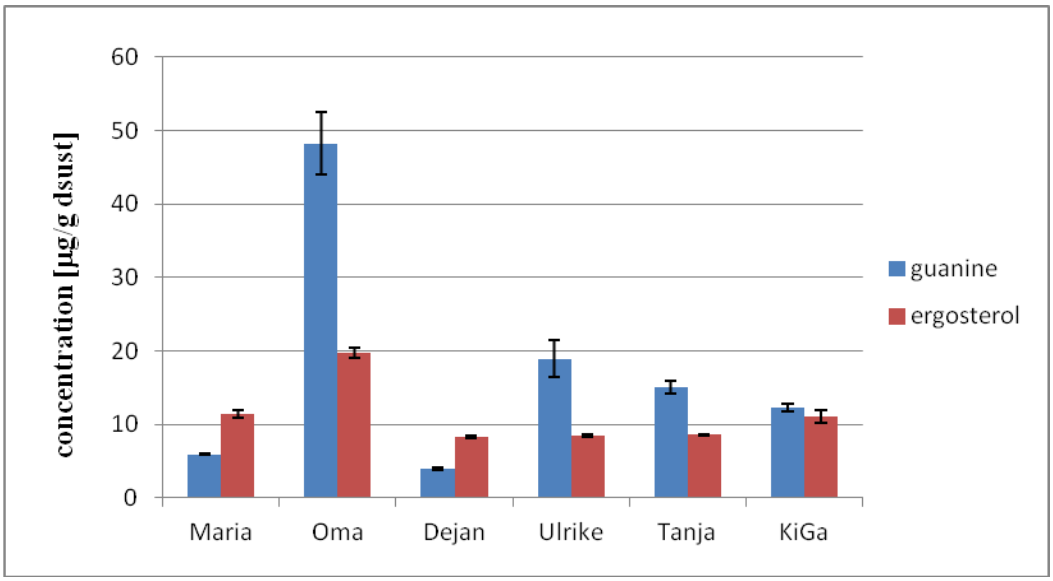

Figure 21: Comparison of guanine and ergosterol levels in house dust

7 Conclusion

This Bachelor thesis presents the determination of allergens, especially guanine and ergosterol, in different house dust samples. Guanine is the major nitrogenous waste products of arachnids and serves as indirect indicator for house dust mites. This is the reason why the literature is mainly focused on the biology of house dust mites but in lesser extent deals with determination of guanine itself, and ergosterol in addition which is regarded as biomarker for fungi and mould growth.

The method for the determination of guanine in house dust was developed using LC/UV and GC/MS. According to these results no risk of house dust mite contamination was observed in all samples. A quick and simple guanine colour test, called Acarex-test, was also performed. This test showed the same trend as the results from LC/UV measurements but two samples were classified to have a low risk of house dust mite contamination.

Determination of ergosterol was more time-demanding but good results were obtained. The amounts of ergosterol found in the different house dust samples are in accordance with other studies in the literature. It can be concluded that no health risk due to moulds or fungi exists in the samples under study.

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