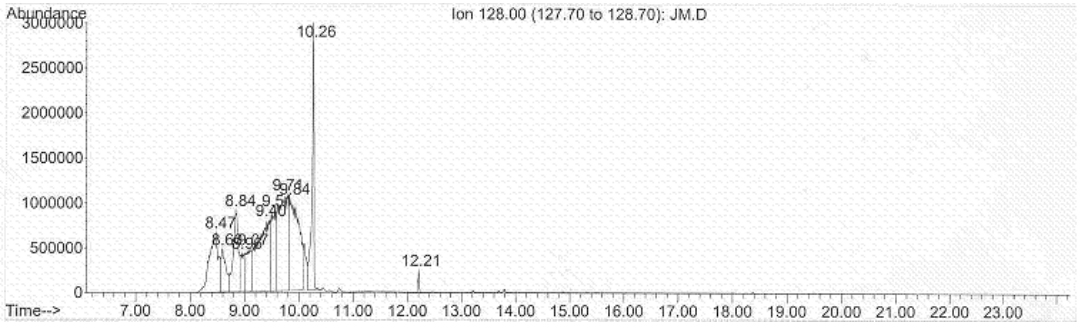
UV-degradation of IPBC in natural water sample



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Abstract

IPBC as a preservative compound for wood furniture has been used for more than thirty years. The degradation of IPBC caused of bacterial biotransformation and UV-light has been considered to be the main reason for IPBC degradation. In our experiments Hg UV-lamp has been used to expose the IPBC solution for UV-light and we found that it degrades immediately. By using GC-MS we found it gives two main products with molecular mass of 112 and 98. The 98m/z is the final product when the UV-light gives enough energy. In a natural water sample only little IPBC degradation product has been found, and it has a molecular mass of 112. This is caused by lower energy that is emitted from natural sunshine compared to the Hg UV-lamp. The little effect sunlight has on IPBC makes it a good outdoor preservative.

Introduction

When wood needs preservation, a lot of thought goes into making a preservative that works effectively against wood degradation caused by weather, fungi or bacteria. At the same time the possible preservatives needs to be tested for toxic specificities, so that it will not have an effect on nature nor on humans. One of the used preservatives in these days is IPBC, which is an iodine containing compound that has little effect on the environment and good preservative specificities.

Testing of this compound has been done at the technological institute in Taastrup, which has in a longer period of time covered the water collecting bottles to protect the IPBC from sunlight. This protection has been executed because the UV in sunlight was believed to degrade IPBC, and thereby giving a false concentration when investigated. The effect of UV on IPBC has been investigated earlier, but with a wage conclusion on how big the effect of UV-light on IPBC is. Therefore it has been in our interest to give an estimate on the effect of UV containing sunlight on IPBC. The estimate will be made by performing experiments on IPBC; first by making a UV-spectrum of IPBC, next by irradiating samples containing IPBC and at the end by running a natural sample through a GC-MS. By using a ruler, naphthalene, the amount of decomposed IPBC can be estimated and the effect of sunlight will then be known.

Theory

IPBC - 3-iodo-2-propynyl butylcarbamate

Many different types of preservatives have been used through time. Many of these have been the best alternative for a long time, although they have some downsides as toxicity. At the same time more than one preservative has been used when optimal protection against fungi was needed. IPBC has since the 1980's been used as a better alternative than other preservatives, because of lower toxicity and better protection against a broader range of wood degrading factors.

Chemical properties of IPBC

IPBC, also called 3-iodo-2-propynyl butylcarbamate, was originally not thought of as an alternative to the already existing products on the market in the 1970-1980's. Instead it was used a mildewcide in paints (Hansen, 1984). IPBC which isn't a naturally occurring compound was registered for the first time in the United States in the 1970's for industrial use as a fungicide (Federal, 1978). At the time it was used in coatings, textiles, paper, adhesives and now a day also in cosmetics. But in the present it is the most used antifungal agent in more countries all over the world (Hosomi, 1998).
IPBC is as the name tells us composed of an iodine propynyl group and a butylcarbamate group, and has the molecular formula $C_8H_{12}INO_2$, see figure 1. The molecular weight of the compound is therefore 281g/mol (Hansen, 1984).

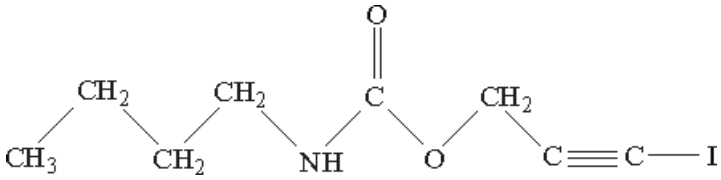
Chemical name: 3-iodo-2-propynyl butylcarbamate, (IPBC) **Molecular formula**: $C_8H_{12}INO_2$ **Molecular weight**: 281g/mol **Appearance**: white crystalline powder **Hydrolysis**: at pH9 buffer **Stability**: 54°C **Mp**: 66-65°C **Bp**: 100°C **Solubility**:<190 ppm in water at 38°C	

Figure 1: shows the simplified structure of 3-iodo-2-propynyl butyl carbamate, and its specificities (Hansen, 1984; Wenninger and McEwen 1993; EPA1997; Blumhorst, 1990; http://apps.kemi.se/flodessok/floden/kemamne_Eng/gif/3-jod-1.gif 21/05/08).

Under standard circumstances IPBC appears as an off-white crystalline compound. IPBC can be dissolved in water, but the solubility is <190ppm when the water is heated to 38ºC (Hansen, 1984) another estimate at the solubility in water at 38ºC is 199mg/l

(reference). In other solvents as polar and aromatic solvents the solubility is high, and in alkyd resins the solubility is medium.
IPBC melts at 65-66 ºC and decomposes at 100ºC. IPBC is found to be stable at 54ºC (Midwest research Institute 1990), but changes in pH can cause it to hydrolyze. When pH is altered to pH 9 IPBC hydrolyzes to PBC, which doesn't occur when pH is 5 (Blumhorst, 1990).

Toxicity
IPBC has been found to be toxic to animals as rats and rabbits. The acute oral LD_{50} was investigated in rats, which gave values at 1580 mg/Kg. A similar value, 2000 mg/Kg, was found in rabbits when the acute dermal LD_{50} was investigated (Hansen, 1984). This values a quite high, which indicates that IPBC is a toxic, but exposure to quite a high concentration is needed for it to be lethal.
Further experiments with albino rabbits have shown that IPBC is irritating and corrosive in eyes, but this is reversible. Experiments performed on rats showed no effects when inhaled. No mutagenic effect has been seen when performing the Ames test, which is an important factor when approving new chemicals.
When using a chemical as IPBC as wood preservative, it's also very important to remember that IPBC could get washed off when it's raining, and thereby end up in the groundwater or in the sea. On this basis another concern has been the toxicity of IPBC to fish (Ward, 1991). Springborn Laboratories has therefore suggested the maximum concentration of IPBC in stormwater to be 67µg/l (Springborn Laboratories Inc., 1990). This concentration has been lowered drastically, when the maximum was set to 1.9µg/l in Canada (Kegley, 2007). This could have been done because further experiments performed in the years 1998-2000, have shown a considerable toxicity on fish (Farrell et al., 1998, Bailey et al., 1999, Office of Pesticide Programs, 2000).
In humans, hypersensitive reactions has occurred, but these were first detected in 1997, and were connected to IPBC in cosmetics in 1999 (Bryle et al., 1997; Pazzaglia and Tosti 1999). This late detection probably appears, because IPBC is first used in cosmetics after many years of use as a wood preservative, where direct dermal contact has been limited.

Use of IPBC
The iodine containing preservative, IPBC, has been used for many years in the protection of wood. In the 1970's and the 1980's other preservatives as pentachlorophenol and sodium salts of tri-, tetra- and pentachlorophenol (NaPCP) have been used. These were very specific, which meant that more had to be mixed to give a good protection. At the same time NaPCP is classified to be more toxic than IPBC (Anon, 1982), making IPBC a possible substitution to lower toxicity. IPBC has shown its protective function against wood destroying fungicides, blue stain, mould and algae. This broad spectrum is what has made it an alternative to NAPCP (Hansen, 1984).
To use IPBC as a fungicide, it has to be dissolved into an organic solvent functioning as a carrier. If the product includes for instance an alkyd, IPBC will be bound and thereby staying in the wood when dried. To obtain the best protection IPBC can be mixed with other materials that can be a part of active protection or make sure that water doesn't enter. At the same time the emulsion should contain the right amount of water, because the solubility of IPBC can change when too much or too little water is used. Too much

water will cause IPBC to crystallize within the water, and thereby not spreading well enough leaving the wood less protected (Hansen, 1984).

Degradation
IPBC is absorbed in soil and degrades to PBC relatively quickly, meaning that it has lost the iodine. When PBC is in environments containing bacteria under aerobic conditions, and at a temperature of 22ºC it degrades further into CO_2. Degradation of IPBC can also happen in sterile soil, where it has been seen that the temperature has great effect on the rate of degradation (Blumhorst, 1990).
Degradation done by sunlight is our main interest and needs more explanation, which is why we have separated it into another topic (Degradation caused by ultraviolet light).

Degradation caused by ultraviolet light
IPBC does, as many other compounds, decompose when irradiated with ultraviolet light. The decomposing is caused when energy overcomes a threshold and thereby breaks the bonds in the compound. We will therefore look a little closer on what ultraviolet light is and what it does to IPBC.

Type	**Abbreviation**	**Wavelength range in nm**
Ultraviolet	UV	$100\ nm \leq \lambda < 400\ nm$
Subtypes		
Extreme Ultraviolet	EUV	$10\ nm \leq \lambda < 121\ nm$
Vacuum Ultraviolet	VUV	$10\ nm \leq \lambda < 200\ nm$
Ultraviolet C	UVC	$100\ nm \leq \lambda \leq 280\ nm$
Far Ultraviolet	FUV	$122\ nm \leq \lambda < 200\ nm$
Middle Ultraviolet	MUV	$200\ nm \leq \lambda < 300\ nm$
Ultraviolet B	UVB	$280\ nm \leq \lambda \leq 315\ nm$
Near Ultraviolet	NUV	$300\ nm \leq \lambda \leq 400\ nm$
Ultraviolet A	UVA	$315\ nm \leq \lambda < 400\ nm$

Table 2: This table shows the wavelength of UV-light, at 100-400nm, and how it's divided into subgroups. (Tobiska and Nusinov 2004)

Ultraviolet light (UV-light) is electromagnetic radiation with a wavelength shorter than that of visible light, but longer than X-rays. UV-light is divided into a range of subtypes that have a broad range of wavelengths, $10 < \lambda \leq 400$ (ISO 2004). Ultraviolet- or UV-irradiances is usually considered to have a wavelength range of $100 \leq \lambda \leq 400$ nm (WHO, 2002). This project is interested in the wavelength range between 200-400nm, where it's believed that IPBC might be affected.
Iodine is a halogen and has bond strength of 228kJ/mol. Some bonds between other atoms and carbon have stronger bonds, C-F bonds (467kJ/mol) and C-H bonds (413kJ/mol) (Hu, 2004). A high number means that the bond between the atoms is strong, and therefore the bond between iodine and carbon breaks fairly easily. The weak bond makes the C-I bond fragile, which means that only little energy needs to be added for the bond to break (Hu, 2004). This energy might come from photons in UV-light, when the molecule absorbs the energy, it transfers enough for the bond to break, which in our case causes IPBC to decompose to PBC. The cleavage leads to the formation of a carbon

radical C• and a release of iodine. Furthermore, the carbon radical can react and form bonds with a hydrogen radical (Cundall, et.,al 1970).
Another factor in the breaking of bonds is the electronegativity. Iodine has an electronegativity of 2.5, which is the same as the electronegativity of carbon. The equality of the electronegativity means that C-I bonds do not have a permanent dipole, and hence easily polarizes (Hu, 2004). This explains the peaks that will be seen when running a sample of IPBC through the GC-MS. IPBC breaks into I + PBC, when the weak bond between iodine and carbon is broken. This will be seen as a peak at /112m z and there
will be some IPBC that doesn't decompose, especially when high concentrations are used, and another peak will be seen at 281m/z. Iodine will not be seen in the data, because it has a negative charge.

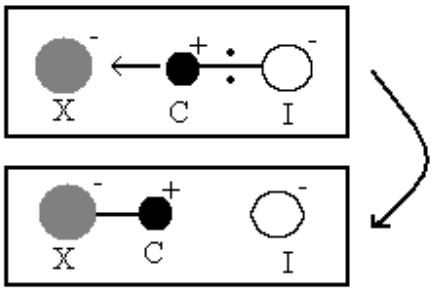


Figure 3: the bond between iodine and carbon will break, when a negative ion with a higher electronegativity closes up on the carbon atom. A new bond between the more electronegative ion and carbon will be made, and the now negatively charged iodine will be free, for bonding with other atoms.

Spectrophotometer
Before beginning experiments on IPBC, a spectrum was made for all the solvents that would be used in further experiments. This was done to see if the absorption of the solvents would absorb light in the range of IPBC, because that would protect IPBC against degradation. This would the lead to a conclusion that could not be used for IPBC in nature, because water is the only other substance in a collected sample from nature.

The spectrophotometer is designed to measure the absorption of a sample in the UV, visible and infrared regions. The lamp used in the spectrophotometer shines light at a specific wavelength onto a dispersion device. A filter is needed though to direct the light onto the sample (Harris, 2007). The intensity, I_0, of the light shined onto the cuvette is usually stronger than the light, I, that has passed through the sample, because of the absorption made by the molecules in the solution. The molecules in a sample will absorb light at a given wavelength, which will show in a diagram. Both the intensity of the incident light and the wavelength are known factors, and the intensity change after having passed the cuvette will be measured by a detector. This will be detected as the absorbance of the sample (Harris, 2007). Before performing any experiments with a spectrophotometer, it should be calibrated by a procedure called zeroing. A standard is chosen, it can be air, water or another sample according to the needs. The standard is then

used to set a baseline value; thereby the absorbance of the following samples will be detected relative to the zeroed substance (Harris, 2007).

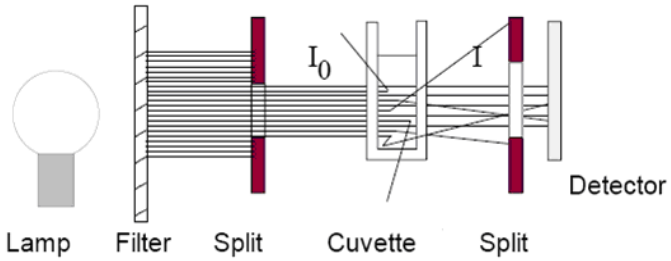


Figure 4: A simplified figure of a spectrophotometer, showing how the light is focused onto the cuvette, and then detected at the end. I_0 is the known intensity of the light before reaching the cuvette. The solution in the cuvette absorbs some of the light, thereby decreasing the intensity (Manual for Introductory laboratory course in molecular biology).

HPLC - High-performance liquid chromatography

The first approach to analyzing IPBC samples was HPLC, but at a very early stage it was proven to be a bad approach. HPLC can only give limited information and more was needed to make sure that IPBC was in the sample. Therefore further experiments were performed using the GC-MS instead, which is why only a little theory on this topic has been written.

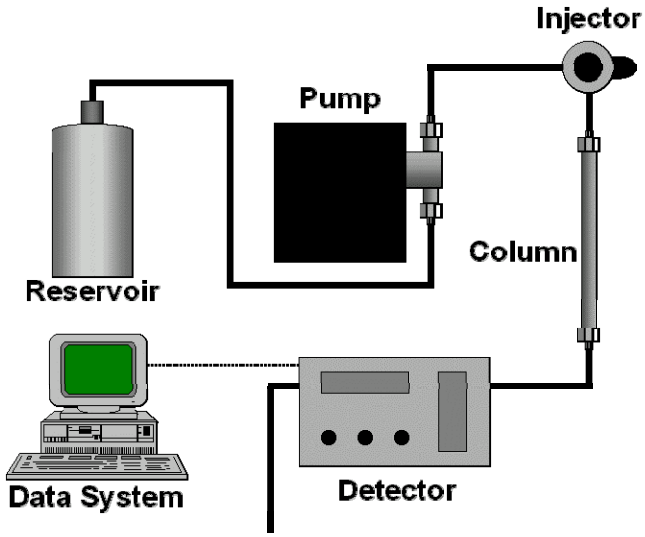


Figure 5: The setup of the HPLC is shown in this simple diagram. When a sample is injected it will run through the column pushed by the pressure. The different components in the sample will then be detected an information will be sent to the computer (http://www.lcresources.com/resources/getstart/generic%20HPLC.gif d. 07/05/08)

HPLC (High-performance liquid chromatography) is a widely used tool in analytical chemistry and biochemistry. The liquid chromatography separates liquid compounds or samples, which are dissolved in the solution (Yu, 2005). HPLC is mainly composed of a pump, an injector, a column, a detector and a computer system with a special program that can analyze the results. When a sample has been injected into the injector then it will

run into the column by the pressure. Different components of the sample have different interactions with the stationary phase, so the injector can detect the different signals. The signals will be shown on the computer, and analysis of signals will make it possible to distinguish the different components of the sample (Yu, 2005).
HPLC analysis is not used for analysis of IPBC, because the IPBC is very hard to detect and therefore the GC-MS, which gives more information, is very useful.

GC-MS - Gas chromatography
GC-MS is a machine used when analysis of a substance is needed. The machine is controlled from a computer, which contains the needed program set up by a qualified person. The program gives the possibility to change different factors as the heat in different parts of the machine or for instance the pressure. Apart from these functions, there is also another very important factor which is how to inject the substance, split-less or all-split, these are chosen from the characteristics of the sample to be injected. When the machine has been set up, then the sample is run through the machine, and the size of ions will be read off in the MS and readings sent back to the computer, which will show diagrams. This will hopefully give the necessary information about the sample to give a reasonable estimate at the compositions of the sample.

When a sample is injected into the system through the heated injection port the sample quickly evaporates, the heat has to be adjusted so that the sample evaporates and doesn't degrade (Hargis, 1988; Currell, 2000). Evaporation of the sample makes it possible for a carrier gas (He, H_2, N_2 or Ar) to transport the sample through the column (Hargis, 1988; Harris, 2007). Analytes are eluted in fairly short time by reaching the required vapour pressure; this is done by heating the wire sufficiently in an oven. When reaching the end of the wire an even more heated detector reads off the substances and sends the information back to the computer.

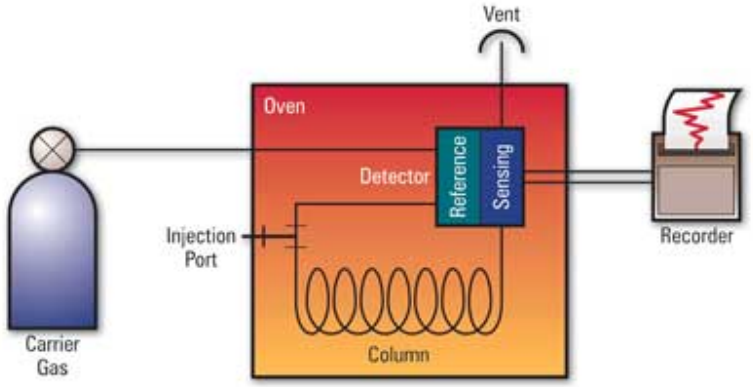


Figure 6: The setup of the GC-MS is showed in this simple diagram. When the sample is injected it will be pushed through the column by the pressure. The column is heated in an oven thereby breaking up the sample. A detector detects these pieces and sends information to the computer (http://images.google.dk/imgres?imgurl=http://www.practicingoilanalysis.com/backup/200207/GasChroma-Fig2.jpg&imgrefurl=http://www.practicingoilanalysis.com/article_detail.asp%3Farticleid%3D352&h=222&w=400&sz=11&hl=da&start=6&tbnid=6dvkNDBsRYl5CM:&tbnh=69&tbnw=124&prev=/images%3Fq%3Dgas%2Bchromatography%26gbv%3D2%26hl%3Dda d. 07/05/08).

The columns used usually have a length of 15-100m, which is why it's coiled (Hargis, 1988), and have an inner diameter of 0.10-0.53mm (Harris, 2007). The different lengths and diameters of the column have different functions. The narrow columns give a higher resolution, but need a higher pressure when operated and can only contain small amounts of sample (Currell, 2000). When a wider column is used the system tends to become overloaded and therefore the stream will be all-split, which only lets small amounts of sample into the mass spectrometer. Open tubular columns are made of fused silica (SiO_2), which is covered with a plastic that can bear high temperatures, up till 350 degrees (Harris, 2007). The cover is used to protect against atmospheric moisture and as a support. Usually these columns are used, although they have a lower sample capacity, because they offer shorter analysis time, have greater sensitivity and a higher resolution. Using copper, aluminium or steel as tubing in gas lines limits entering of air to the system, and also the release of contaminants is limited (Hargis, 1988; Harris, 2007).
In front of the column a 3-10m long guard column, and a retention gab is found. These two columns have different purposes although they are similar. The guard column hinders contaminating non-volatile substances from accumulating in the chromatography column. The end of the guard column is cut of when changes to the usually regular peaks are detected; hereby the impurities are cut off. Separation of solvent from solute is done when the solvent is evaporated before entering the chromatography column, this is done by the retention gab and an enhancement of peaks is then achieved (Harris, 2007).
Temperature and pressure are other important factors, for instance when separating the components of the sample, and also for the retention time (Currell, 2000). Temperature is raised when running a sample hereby raising the pressure and lowering the retention time. Pressure has practically the same effect on the retention time, because altering the inlet pressure decreases the retention time. An increase in pressure also increases the flow of the mobile phase. At the end of a run the pressure can be lowered quickly, thereby saving time by doing less preparation before the next injection. At the same time changes in pressure is very valuable for samples that will not tolerate high temperatures.
As mentioned three different gases with different specificities are used in the GC (Currell, 2000; Harris, 2007). N_2 is for instance better than He if a low detection limit is needed in a flame ionization detector. The carrier gas can also be changed to achieve an optimal flow rate, which can be done by using N_2. H_2, although, is very useful for quick separations of the sample and can be run faster than its optimal velocity without doing big harm in resolution. A huge problem when using H_2 is that explosive mixtures form in air. Other but smaller problems, is breaking down of vacuum pump oil in the detector, as well as it can start catalytic reactions when it mixes with unsaturated compounds in a sample (Harris, 2007).
Using a carrier gas that separates well in the column and addition of another gas in between the column and the detector improves the poor detection performance caused by narrow columns. Traces of organic compounds and O_2, as well as H_2O shouldn't be found in the used carrier gas, because they may damage the system by degrading the stationary phase found on the inside of the column. This is also why high quality pure gas is used in the machine (Harris, 2007).

Different detectors are used in gas chromatography; thermal conductivity detectors (TCD), flame ionization detectors (FID) or electron capture detectors (ECD). Focus is set on ECD, while it's used in our machine.
ECD is very sensitivity and has a detection limit comparable to single ion monitoring, and is especially sensitive towards halogen containing samples, as well as to nitro compounds and organometallic compounds (Hargis, 1988). In contrary it's insensitive to ketones and alcohols (Harris, 2007). The gas carrying the compound of interest can be either the carrier gas used in the column or a "make-up" gas that has been added between the column and the detector. These gasses have to be either N_2 or 5% methane in Ar, and have to be free of moist, because it will make the detector less sensitive. A foil with radioactive ^{63}Ni emits β-rays, thereby ionizing the gas and releasing electrons (Hargis, 1988; Currell, 2000; Harris, 2007). An anode in the detector attracts the electrons making a current. When high affinity analyte enter the detector electrons are captured, which makes a change in the current (Hargis, 1988). These changes are corrected by the detector by adjusting the frequency of voltage pulses between the anode and the cathode (Harris, 2007). These changes are registered and sent to the computer where they can be seen.
Another detector used in relation to GC is the mass spectroscopy (MS). MS is connected to the GC by capillary columns that can connect the two directly. These capillary columns carry the gas at low gas flow rates into the mass selective detector. When separation between carrier gas and analyte is needed, a jet separator is put to use, which sends the gas into the MS through a small tubular input (Currell, 2000). The carrier gas, mainly consistent of small molecules, diffuses out of the stream and is sent off, while bigger molecules are too heavy and will enter the MS. A beam of electrons are shot at the entering analyte from a hot filament, when using electron ionization (EI) (Harris, 2007). The beam of electrons causes the molecules to fragmentize. Fragmentation occurs because the energy of the electrons exceeds the energy needed to ionize (Currell, 2000; Harris 2007). The kinetic energy of the electrons, usually at 70eV, can be lowered, thereby giving a higher abundance of the molecular ions when needed. Lowered kinetic energy also causes less fragmentation that makes comparison of the fragments in the library more difficult (Harris, 2007). The fragments are detected and their mass information is sent to the computer, which displays the result.
Another method of detection, chemical ionization (CI), can also be used when applying MS. This method though isn't used in our equipment, which is why isn't described further.

Materials and Experiments

This project contains an investigation on the effect of UV-light on IPBC. To be as thorough as possible an UV-spectrum was made on IPBC in different solvents, which would be used when UV-degradation would be performed. At the same time the UV-spectra of the solvents themselves would need to be determined, so that wavelengths at which absorption happen could be determined. Having done these the degradation of IPBC could commence. These experiments would be performed with a UV-lamp, and thereby shining artificial light on it, before running some samples in sunlight. All of these samples would be run through a GC-MS to determine if and how much UV-degradation has occurred.

For a more detailed description of the method and the experiments performed go to the appendix.

Materials & Chemicals

- 2.148µg/ml IPBC in methanol
- 99.8% Dichloromethane (CH_2Cl_2) Lab Scan, Ireland
- 99.9% Methanol (CH_3OH), Lab Scan, Ireland
- 98% Naphthalene ($C_{10}H_8$) Aldsich-Chemie, West Germany
- Acetone ($(CH_3)_2CO$) (Just for cleaning purposes)
- Quartz cuvette for UV-spectrum, Pelkin-E (3ml)
- Multipurpose Recording Spectrophotometer MPS-2000, Shimadzu, Japan
- UV-lamp, ORIEL-Arc lamp ignitor, Arc lamp power supply, and Hg lamp, USA
- GC: Agilet 6890 system, MS: Agilet 5973 MS Detector, USA
- LC-MS Finnigan LCQ (TM), DECA, ThermoQuest, USA
- Balance, Mettler AE 200, Mettler-Toledo, Denmark
- SPE
- 50ml beaker, VWR (VWR1213-1169) Borosilicate glass 3.3
- 10ml volumetric flask, MBL England 10ml ±0.025ml in 20ºC, ISO 1042, and Hirschmann EM techcolor Germany 10ml±0.04ml in 20ºC
- Pipette
- 500µl syringe, Hamilton-Bnaduz, Switzerland, Gastight ® 1750
- Parafilm M, Pechiney Plastic Packaging, USA
- Vials (32 x 11.6mm)

Experimental setup

Our sample of IPBC is contained in methanol, with a concentration of 2164.8µg/ml. This solution will be mentioned as IPBC in the following descriptions.
Quartz cuvettes were used in all experiments, although prior experiments had used pyrex cuvettes (Lee, et al., 1990). This was done to limit the absorption of UV-light that usually occurs when glas cuvettes are used.

UV-spectrum

IPBC was diluted in dichloromethane with a factor of 30, giving the final concentration of 72.16µg/ml. The machine was then set to shine UV-light with wavelengths of 200-500nm onto the sample. Because IPBC as mentioned earlier is in methanol, and diluted in dichloromethane, further experiments were done to find the absorption range of IPBC itself. The absorption range of dichloromethane was found by making a pure sample of dichloromethane, and let UV-light in the same range shine onto the sample. These results would then be subtracted from the results of IPBC in dichloromethane, which will give the absorption range of the compound, IPBC, and methanol.
Making an UV-spectrum of methanol diluted in dichloromethane with a factor 30, will simulate the concentration of methanol found in the first experiment. The UV-spectrum would then again be subtracted from the spectrum of the first experiment containing IPBC, leaving the absorption range of pure IPBC. Pure IPBC absorbs at a wavelength of around 275nm.

Knowing the absorption range of pure IPBC, another experiment would be done to find the absorption range of water. When IPBC is tested at the technological institute in Taastrup, the sample is collected in water. If water absorbs at the same range, then it will be protecting IPBC from getting degraded by UV-light. Hence, water was diluted in dichloromethane with a factor of 30, which would give the absorption spectrum of water needed to compare with the first test performed.

UV-degradation
IPBC in dichloromethane with a concentration of 216.48µg/ml was made. The prepared sample was put into a quartz cuvette, before shining UV-light at all ranges onto the sample. Another team of scientists have previously made an experiment concerning the degradation of IPBC made by UV-light (Lee, 1991a). They had observed degradation of IPBC after it had been exposed to the UV-light of sunlight for 108hours. We would use an UV-lamp instead, which is much more powerful and has a broader range of wavelengths. Our first sample of IPBC was therefore exposed to the lamp for only 160min. The sample would then be put into vials before being run in the GC-MS. This first sample showed a strong degradation, leading to further experiments with a shorter time of UV-light exposure. To find the time at which IPBC degradation occurs, samples were run after UV-light exposures in; 10, 20, 30, 40, 50, and 60min. This was then further extended with the addition of water that acted as a filter for UV-light.

Gas chromatography
Standard samples containing IPBC in dichloromethane, and degraded samples were run in the GC-MS. The GC-MS was used to show the standard peaks of IPBC, and then the peaks of fragments after UV-degradation. There had been some trouble with readings under 200ppm, which made adjustment of the method necessary. These adjustments were made from knowledge about the solvent and the nature of IPBC, and readings could then be performed at 1ppm. The start temperature of the oven in the apparatus should be set to 35°C, when dichloromethane is used as solvent. This temperature would rise with 20°C/min until 300°C were reached. Another factor to detect ppm levels, were to inject a large quantity. We therefore used split-less, and injected 5µl, instead of the standard 1µl, giving us a clearer peak. Split-less had to be combined with single ion monitoring (sim) instead of scan. This is a change in the way the MS reads the ions, instead of giving a spectrum of all ions it will only detect and show selected ions. We had chosen 281m/z and 112m/z, because 281m/z is the molecular weight of IPBC, while 112m/z is the molecular weight of IPBC without iodine. 281m/z should be the peak with the highest abundance in standard samples and 112m/z should have a lower abundance, this is opposite when IPBC has been exposed to UV-light and degradation has occurred.

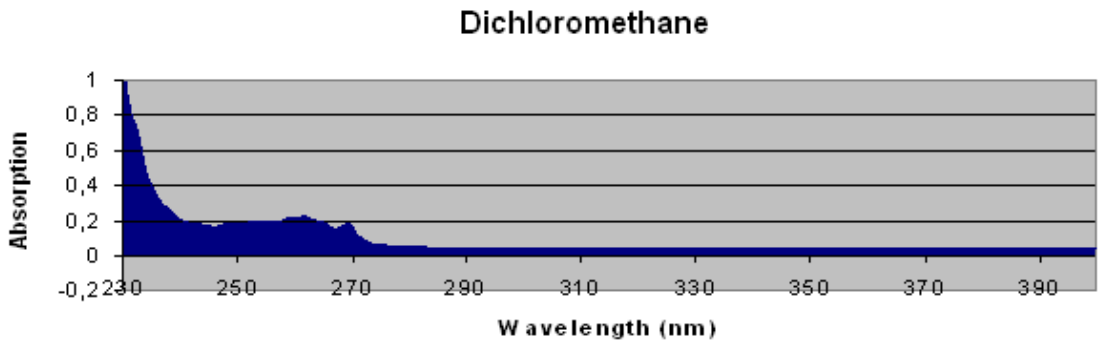
Adding an internal standard
A known concentration of naphthalene was added to a solution containing a known concentration of IPBC, which was diluted in dichloromethane. Results of the first readings of the GC-MS were used to enhance the internal standard, so that the ratio between naphthalene and IPBC would be fairly close, making it easier when unknown concentrations of IPBC were to be estimated. The internal standard would later be used when running the sample from nature in the GC-MS. The naturally occurring

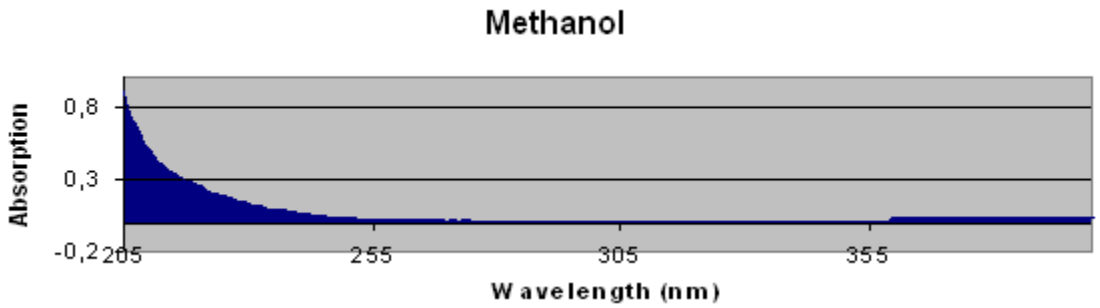
concentration of IPBC in water, when collected, has a concentration of 0.5-2.5ppm (Teknologisk institute, Taastrup). Such low concentrations would without an internal standard be hard to estimate, because readings of the GC-MS are a little unstable. The instability has little importance when high concentrations are estimated, but they are important in small concentrations, hence the internal standard. In samples with unknown concentration of IPBC, a known concentration of naphthalene will be added, and an estimate on the concentration of IPBC in the water can be made, because of the internal standard.

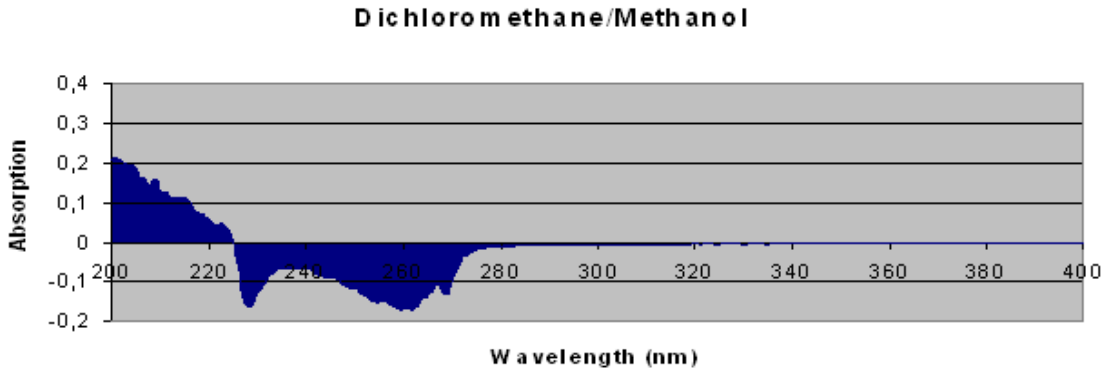
Natural sample
Water containing an unknown concentration of IPBC is collected from the technological institute in Taastrup. This water has been exposed to natural sunlight, which is why it's interesting to see the level of IPBC and the level of degraded IPBC in the water. The sample is in water, and therefore has to be extracted before running the sample in the GC-MS.
The method used to extract IPBC from water is called solid phase extraction (SPE). The water containing IPBC is poured into a syringe containing a solid phase. The structure of the solid phase captures long carbon chains, as IPBC, and lets the water pass through. All that is not captured in the solid phase is collected in a test tube, which is emptied when full, and then reused for further collection of water. The water runs slowly through the solid phase, and is therefore sucked through the syringe by low pressure. To get IPBC out of the solid phase, an organic solution, such as methanol, is used. Methanol containing IPBC is collected in a beaker and naphthalene is added as an internal standard, before the sample is put into a vial and run in the GC-MS. The known concentration of naphthalene makes it possible to estimate the concentration of IPBC or of its fragments in the sample.

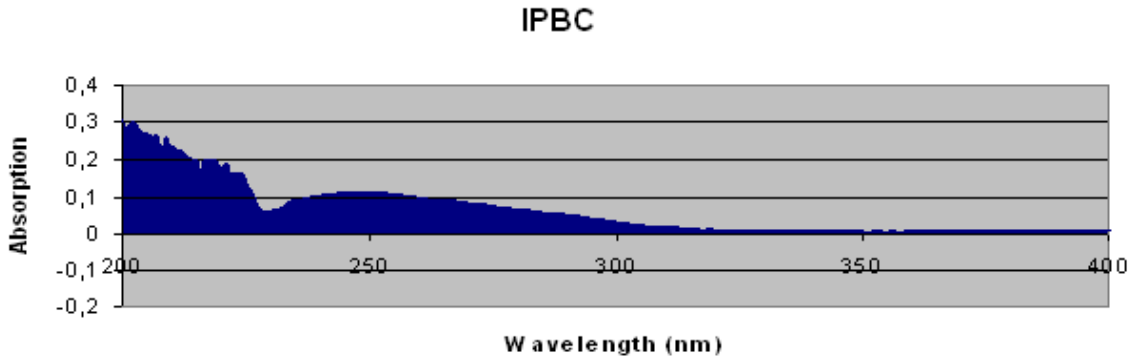
Results

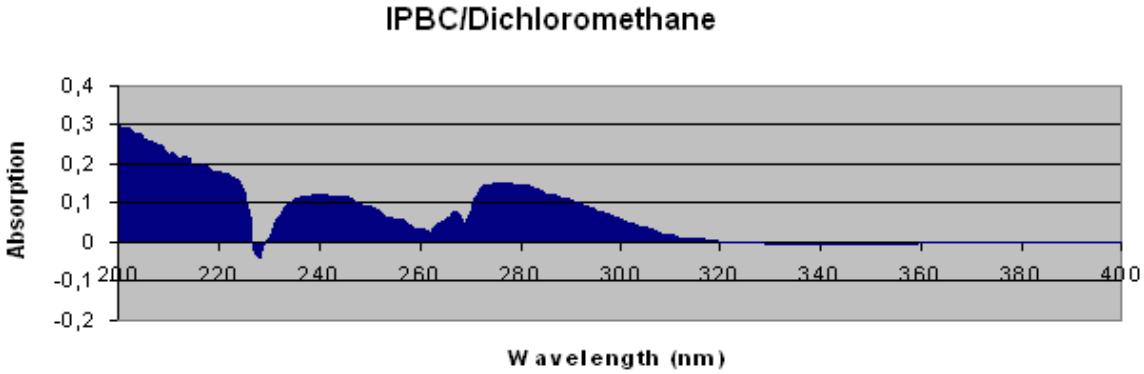
UV-spectrum



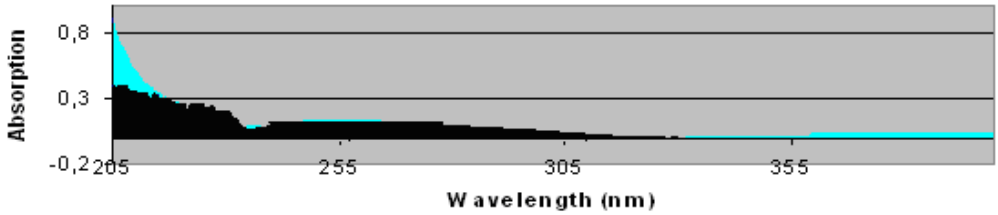

Methanol
Absorption
0,8
0,3
-0,2
205
255
305
355
Wavelength (nm)


Dichloromethane/Methanol
Absorption
0,4
0,3
0,2
0,1
0
-0,1
-0,2
200
220
240
260
280
300
320
340
360
380
400
Wavelength (nm)


IPBC
Absorption
0,4
0,3
0,2
0,1
0
-0,1
-0,2
200
250
300
350
400
Wavelength (nm)



The curve shows the wavelength of IPBC; normally it has absorption at 275nm (Reference ???). Because the IPBC is a solution with methanol, so the absorption of methanol has been test as well, then the absorption of IPBC can be defined by subtract the methanol signal. (see picture below). It gives a value at 255 or 260. Because methanol is not pure, so the solution also contains some water and water will also absorbance the UV light.

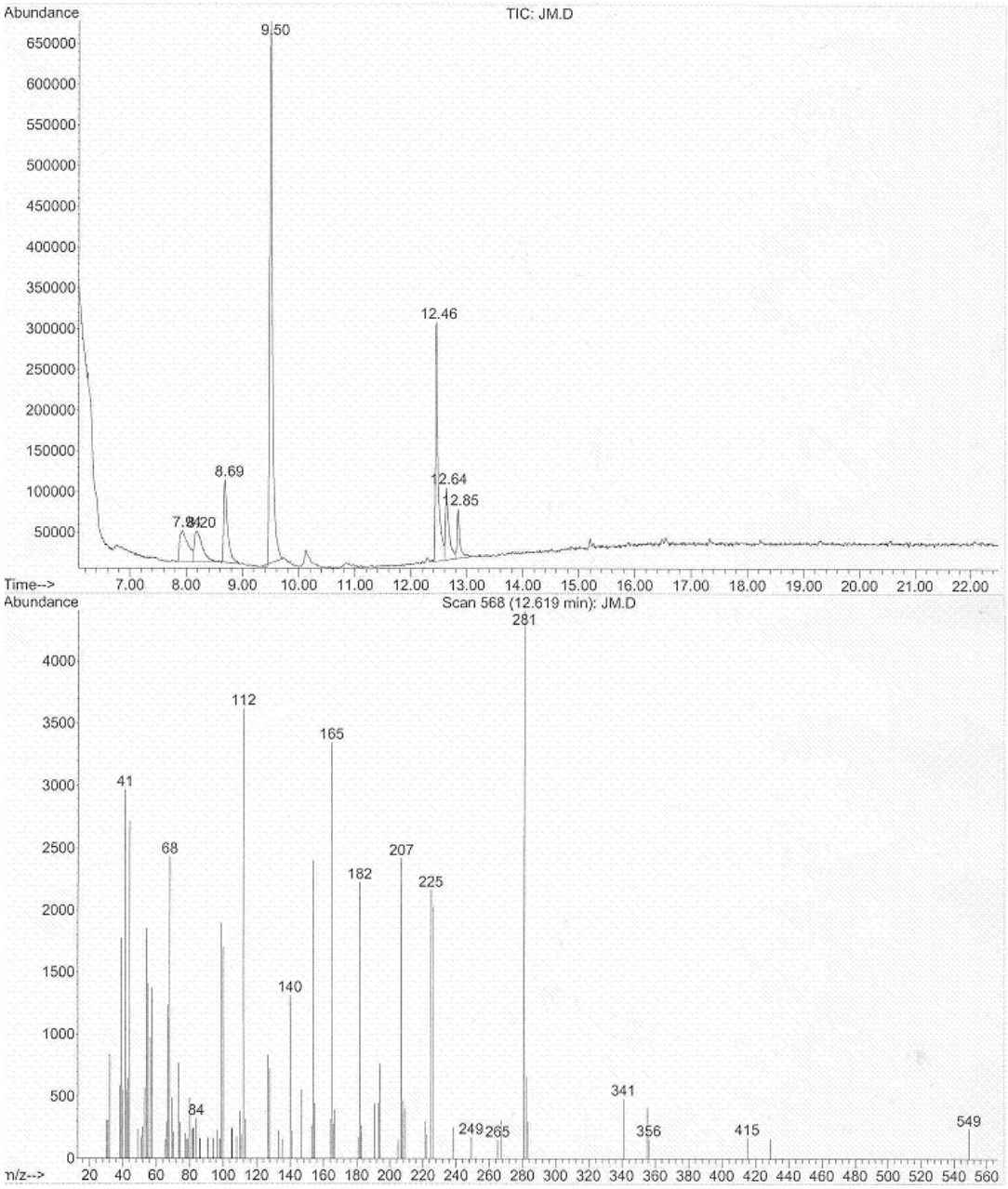


IPBC and IPBC degradation

Capacity = ratio of IPBC in natural sample / ration of IPBC in standard solution
= (area of IPBC in natural sample/ area of naphthalene in natural sample)/ (area of IPBC standard/ area of naphthalene standard)

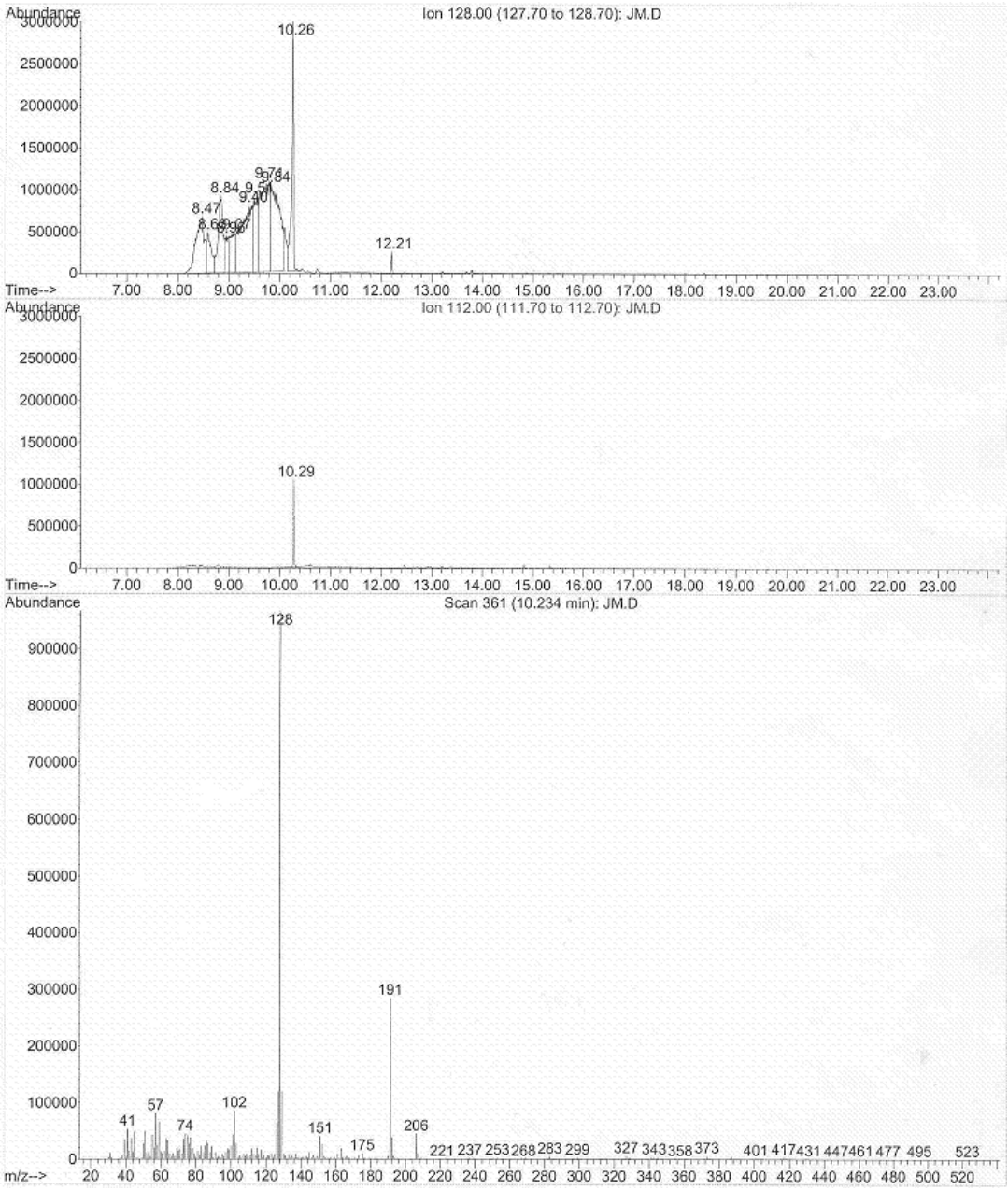
Compounds Name	Area	Capacity
Naphthalene(in standard)	23528673	0,182/0,253 = 0,72=72%
IPBC(in standard)	3803258+2153047=5956305	
Naphthalene (in natural sample)	76105226	
IPBC (in natural sample)	13875427	

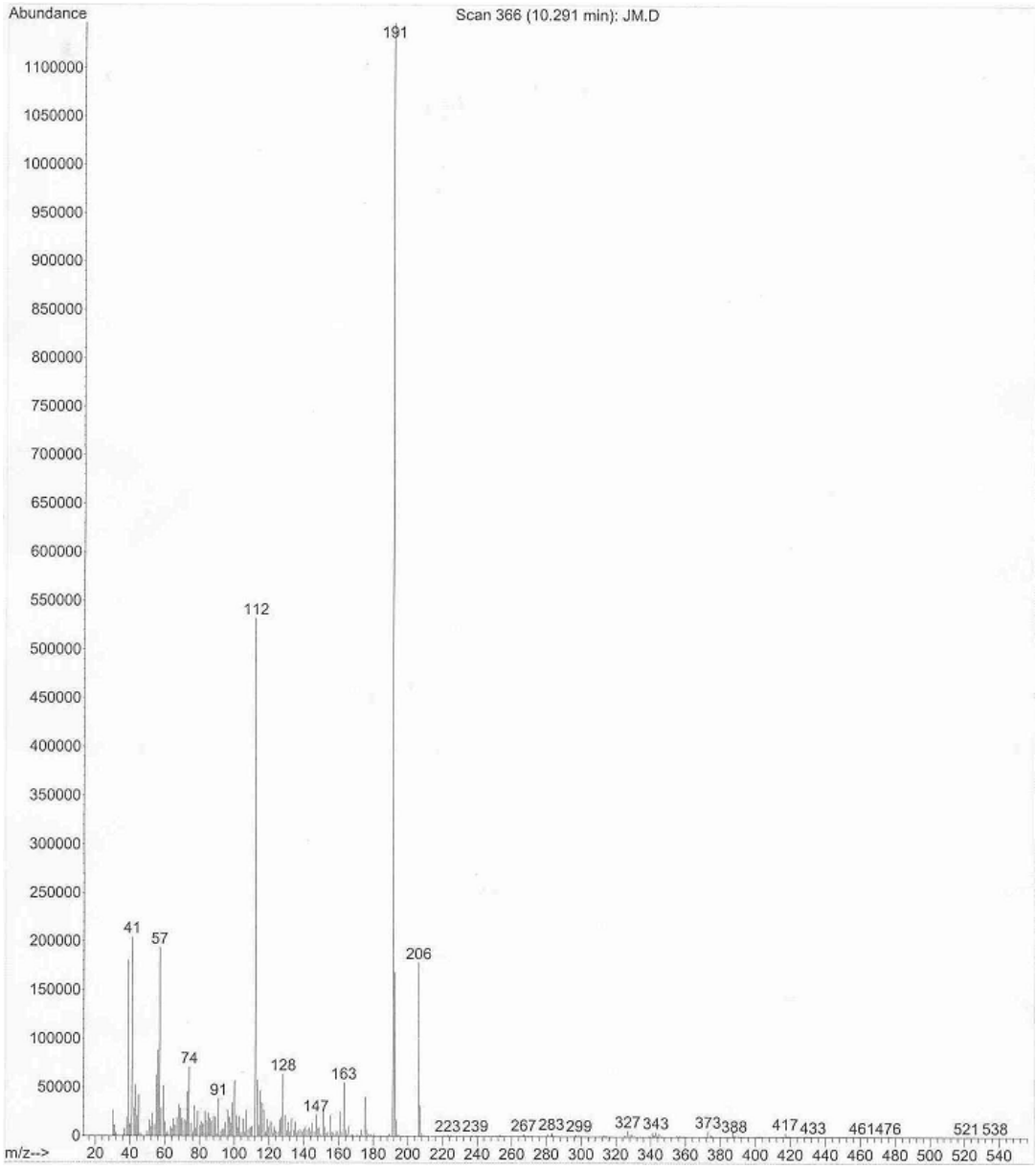
The capacity is much too high, and must have been caused by some mistake in the equipment or by another source of error. Therefore further calculations on this are not performed.



The standard: 10µg/ml naphthalene in 100µg/ml IPBC.

From the chromatography four main peaks are found. The first one at 9.50min is the naphthalene, which has a mass of 128m/z. At 12.64min and 12.85min we found peaks with the following masses 281m/z and 112m/z. These are accordingly IPBC and its fragment.


Abundance
Ion 128.00 (127.70 to 128.70): JM.D
10.26
12.21
Time-->
Abundance
Ion 112.00 (111.70 to 112.70): JM.D
10.29
Time-->
Abundance
Scan 361 (10.234 min): JM.D
128
191
57
102
41
74
151
175
206
m/z-->

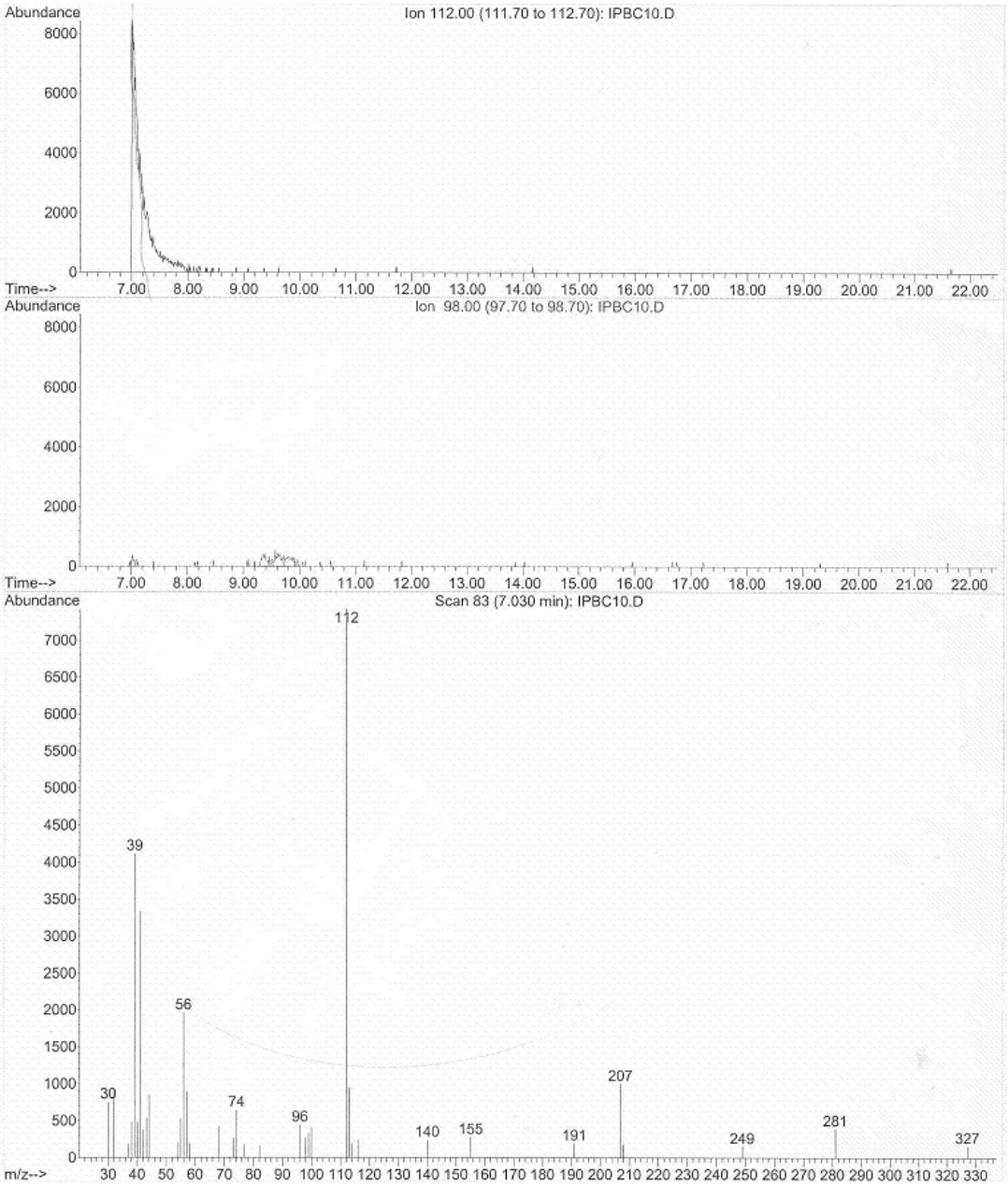


Natural sample: 5µg/ml naphthalene in 50ml of natural water extraction.

This is the result from natural sample; the water sample has been exposed in nature for one week. After extraction, 5µg/ml naphthalene has been added, as an internal standard. However the peak of naphthalene has moved from 9.50min to 10.26min, which may be caused by pollution in our equipment. Little IPBC and degradation fragment of IPBC has been found at 10.29min. The fragment 191m/z is considered to be pollution.

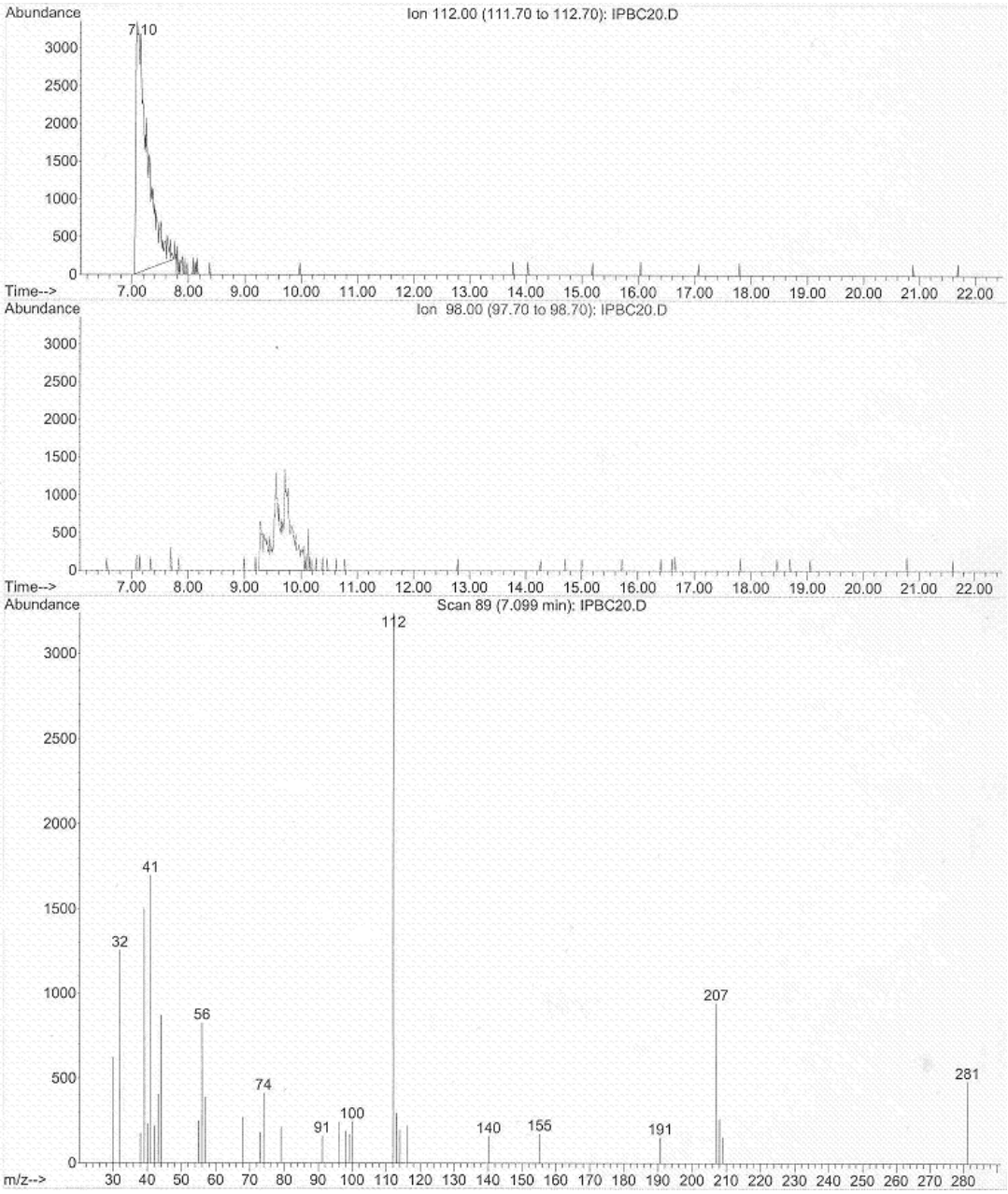
IPBC with water filter

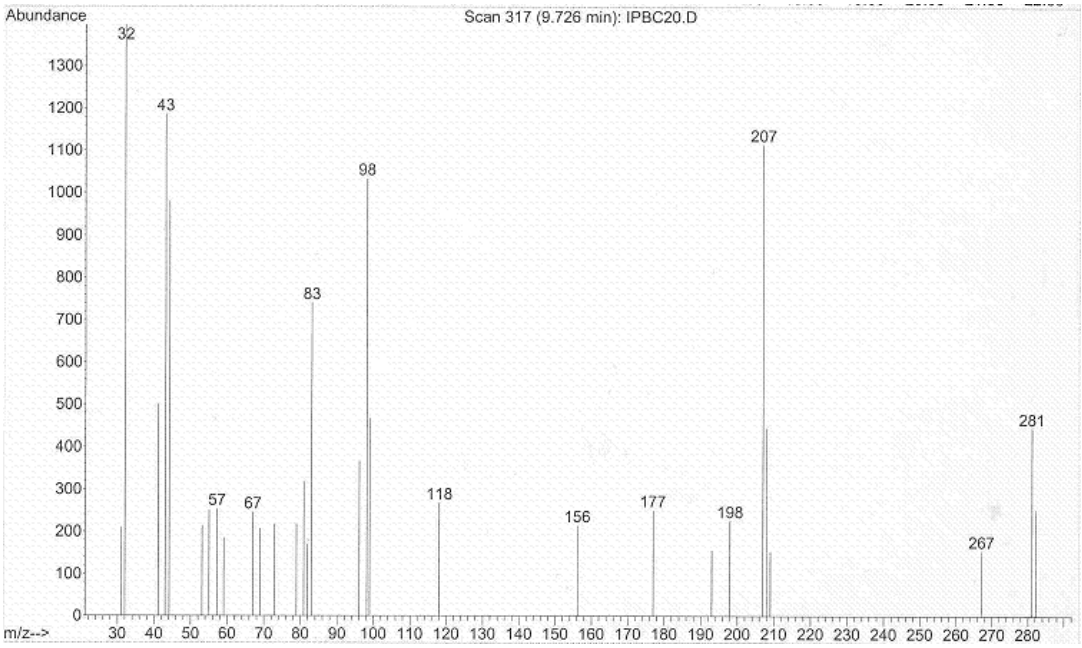
IPBC with water as a filter has a concentration of 100µg/ml. After exposure of the sample to a mercury lamp, the sample has been test by GC-MS. By the mass spectrum, we found that IPBC has almost degraded and becomes fragments with a molecular mass at 112. Only a little amount of fragment with molecular mass at 98 has been detected.



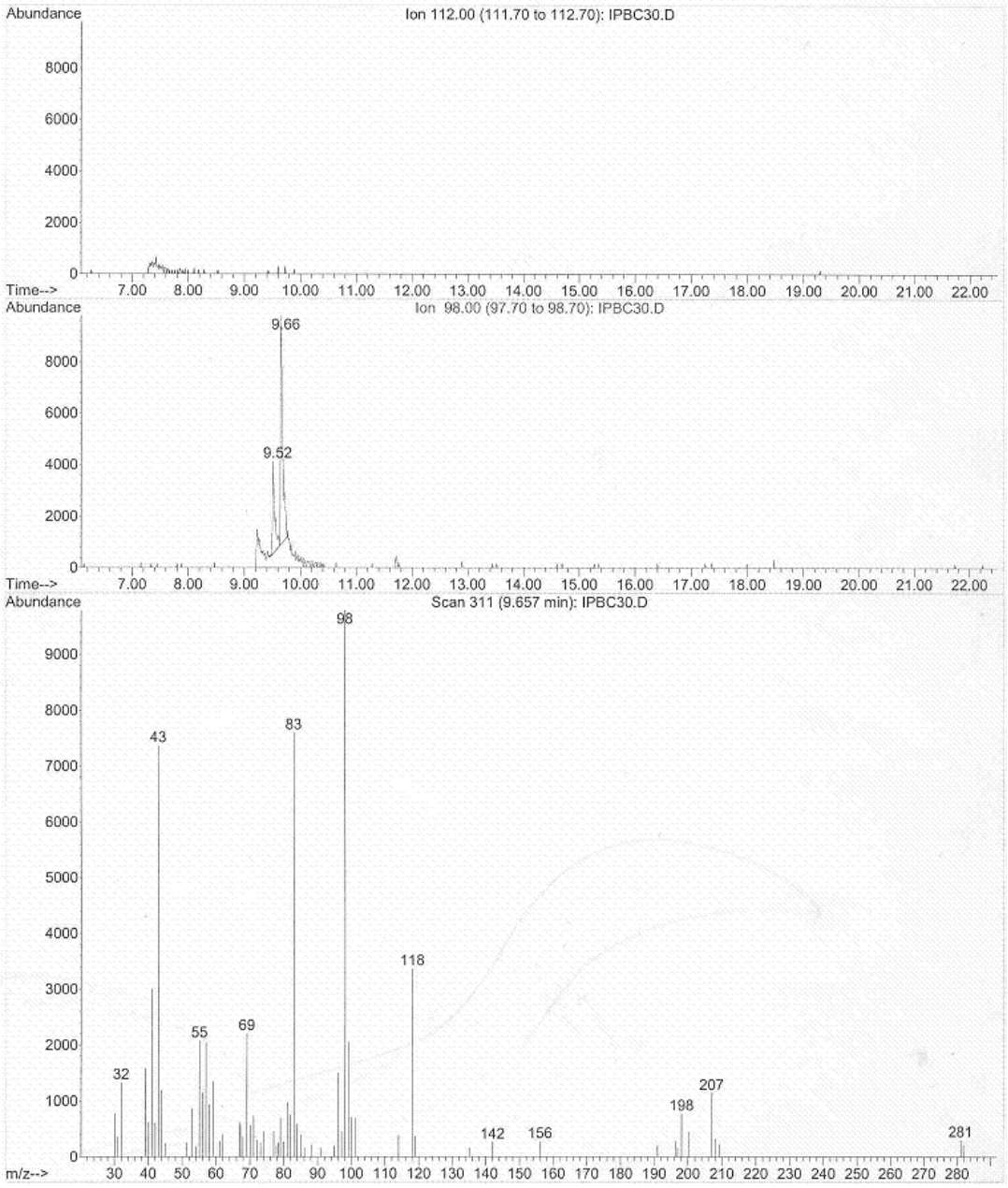
10

The IPBC sample had been exposed to UV-light for 10min, and showed a clear peak in the diagram. The signal had a retention time of 7.02min and a peak height of 7914. When looking at the mass spectrum, the peak was analyzed to be consisting of mainly 112m/z, although other fragments were seen, they were of no importance.

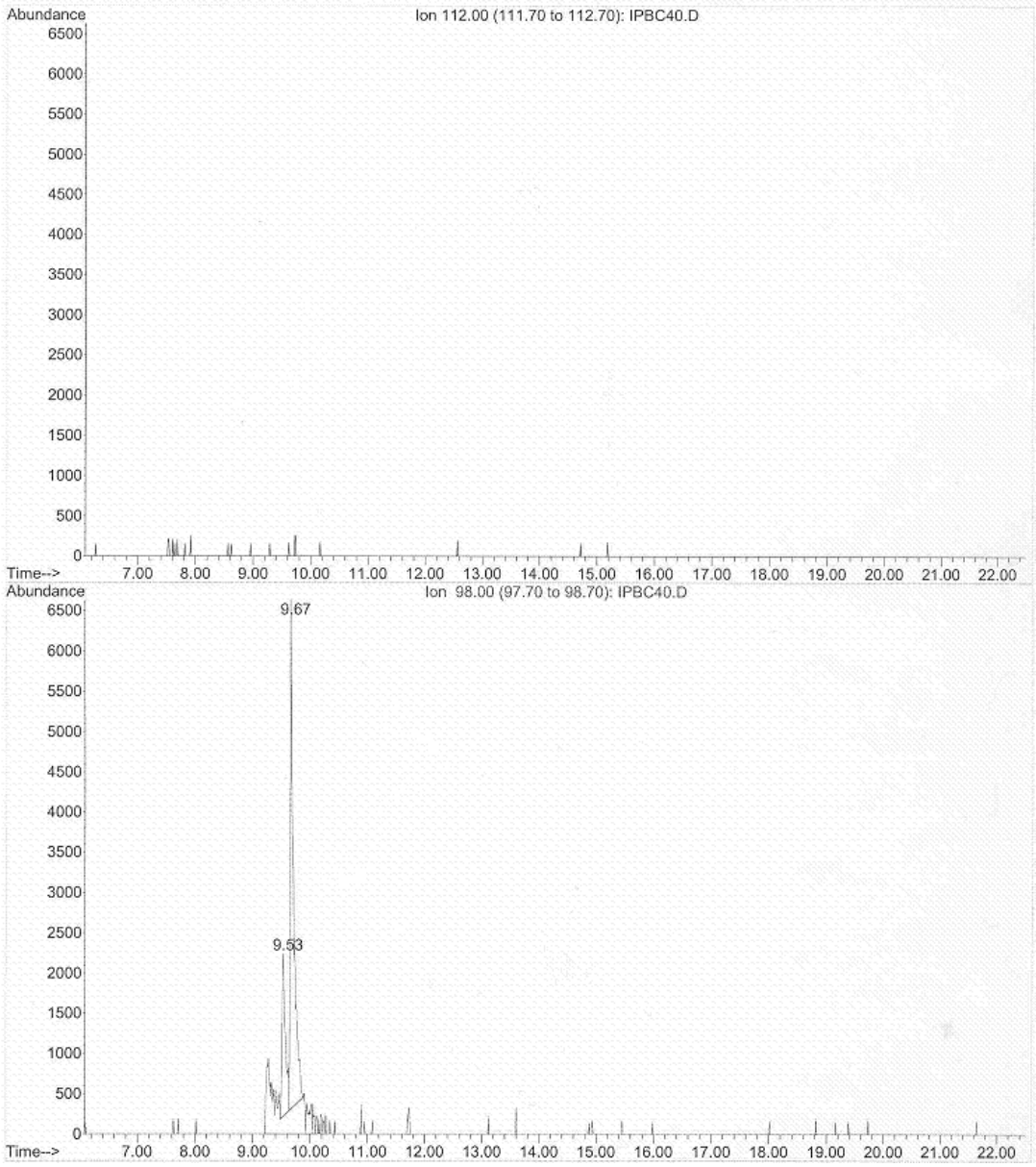

Abundance
Ion 112.00 (111.70 to 112.70): IPBC20.D
7.10
Time-->
Abundance
Ion 98.00 (97.70 to 98.70): IPBC20.D
Time-->
Abundance
Scan 89 (7.099 min): IPBC20.D
112
41
32
56
74
91
100
140
155
191
207
281
m/z-->



After 20min of UV-exposure, a peak with the height of 3228 appeared after 7.10min. Once again the mass spectrum showed that 112m/z was the main fragment in our sample, but the concentration had decreased. The decrease in concentration of 112m/z, is probably caused by further fragmentation. Further more the peak was delayed with 0.08min compared to the sample that had been exposed to UV-light for 10min.



When the sample had been exposed to UV-light for 30min a significant change was noticed. Instead of just a single peak, a triple peak appeared and much later than previously seen. The triple peak was seen after 9.66min which is a delay of 2.56min compared to the peak seen after 20min of exposure. The mass spectrum now showed a new main fragment of 98m/z. The altitude of the peak for 98m/z was 8192, which before 30min of degradation was unrecognizable for the program. Though the mass spectrum didn't show a peak for 112m/z any more, which could be because the UV-light has added sufficient energy to the sample for 112m/z to fragmentise even further and become 98m/z or other fragments.



After 40min of exposure to UV-light, the main peak of the triple peak appeared after 9.67min, which was only a delay of 0.01min. Therefore the degradation of the sample has finished, meaning that the final main degradation product has been reached. The peak height of the highest peak was 6250, which shows that more fragments with smaller molecular weight have been made, though 98m/z is still the main fragment.

The spectrums for 50min and 60min of exposure to the UV-lamp are not shown, because there are no important changes. Though we will still comment on the results, as follows; exposure to UV-light in 50min gave the same recognizable triple peak after a retention time of 9.67min. The highest peak had a height of 8619 which is an increase compared to the result seen after 40min of degradation. When making a mass spectrum, the main fragment was once again 98m/z and still no 112m/z could be detected in the sample.

After 60min of UV-light exposure, the triple peak was seen after 9.66min, which is 0.01min quicker than the last two runs in the GC-MS. This change isn't of any importance, but rather a variation in the machinery. The height of the peak was 8863, which was an increase, although a variation is seen, one could say that the concentration of 98m/z is quite stable.

From these results we see that 98m/z must be the main final product of the degradation, because no change of importance is seen in the retention time, or in the peak height, and because other peaks do not appear. The peak height varies, but is almost stable except from one deviation occurring after 40min of degradation that could be caused by variations in the machinery. All of this shows us that the fragment, 98m/z, is the main degradation product that will be seen when IPBC has been exposed to mercury UV-lamp for more than 30min. When exposed for less time, the 98m/z peak isn't detected, though another peak at 112m/z is found, which is a fragment of IPBC missing iodine and carbon tail. The reason why 98m/z isn't detectable before UV-light exposure for 30min, can be because it needs more energy to fragmentise 112m/z to 98m/z, than it takes for 281m/z (IPBC) to fragmentise to 112m/z. The difference in fragmentation and energy needed is caused by the bonding specificities of IPBC. The 98m/z peak seen in the mass spectrum, is not seen in the natural sample, which might be because sunlight doesn't have the energy that is necessary for further bonds to break, and degradation then stops when IPBC has lost iodine and the $(CH_2)CH_3$ chain.

Discussion

IPBC degradation in water sample

The UV spectrum shows that IPBC has the highest absorption at 275nm. The degradation product of IPBC depends on the exposure time and the intensity of the light shined onto the sample. The main degradation production of IPBC is 112m/z in standard and in natural sample. In prior work done by Lee et al. 1991b, the main degradation product has a molecular mass of 155m/z which indicates the loss of iodine (281m/z-127m/z + H= 155m/z). We didn't find this, which might be caused by the intensity of the light emitted from the lamp. Too much energy added, will break the iodine bond that is the easiest break in the IPBC molecule, and break further bonds immediately and we will then only see the 112m/z peak. IPBC is a long carbon chain, containing some oxygen, nitrogen and iodine, therefore exposing it to a UV-lamp will give many possibilities for fragmentation. The more time the sample is exposed the more energy will reach the sample thereby breaking further bonds.
Another reason why we don't see the 155m/z peak, can be because we used a quartz cuvette, while Lee et al., 1991b used a pyrex cuvette. When using a quartz cuvette, no UV-light is absorbed by the cuvette letting all the energy reach the molecule, but using a glas cuvette decreases the UV-light, and thereby the energy let through, and will protect IPBC against degradation.

The main final product of UV-degradation in the water sample is 98m/z. It has been considered as the product of further degradation of the fragment 112m/z. The 98m/z fragment isn't seen in the natural sample. This is because the intensity of the mercury

lamp is much higher than the intensity of the sunlight in nature, so the natural sample does not get enough energy for further degradation. Therefore we only expect to see 112m/z as the degradation product of sunlight. Though, we need to remember that the sample will not be sterile after a week of exposure to the nature. It is almost certain that bacteria will be found in the water sample collected, thereby we know that degradation caused by bacteria should be considered. The degradation product of bacteria is the 112m/z fragment, (Cook et al., 2002) which makes it difficult for us to know whether the 112m/z fragment is caused by UV-degradation or by bacteria degradation. To determine the cause of degradation further experiments will be needed, as mentioned in the perspective.

IPBC degradation in natural sample

We use splitless method and the injection volume is 5µl in GC-MS, and get the degradation product of IPBC as a triple peak in chromatography, with a mass of 112m/z. Compared to the standard we believe that there will only be a little amount of IPBC degradation product in the natural sample.

Our GC-MS detection limit for IPBC is then found to be 1ppm. However, SPE method has been used successfully for IPBC extraction and up concentration of the sample has altered the concentration above 1ppm level.

To extract the sample by the SPE method, methanol was used, which was different from the solvent, dichloromethanol, used in all other experiments, but this is considered and is not believed to have a big effect on the result.

Naphthalene is used as an internal standard. In the standard sample both naphthalene and IPBC have a known concentration. In the natural sample, which has an unknown concentration of IPBC, naphthalene of a known concentration is added. Because the ratio between the two compounds will not change, then the unknown amount of IPBC in real sample could be calculated. Problems with the readings of the machine, has given areas under the peaks that cannot be used for further calculations, therefore the amount of IPBC is not calculated.

Conclusion

We can on the basis of our experiments and the research made prior to the experiments, conclude that IPBC is definitely affected by UV-light. When very intense UV-light is shined onto IPBC it degrades to the fragment 112m/z and finally gets the further degradation product at 98m/z. Though when exposed to natural light that is less intense, then UV-degradation occurs, but the energy is not enough for IPBC to degrade further than 112m/Z, so we do not get a further degradation product as 98m/z. As sunlight is much weaker than a Hg UV-lamp, the IPBC degradation is limited. This makes it a good preservative, although further experiments should be performed to give an exact estimate on the degradation caused by sunlight compared to that done by bacteria degradation.

Perspective

In producing this report, a big effort has been put into the experiments. There has been a lot of trouble reading the wanted concentrations on the GC-MS machine, which has limited our time noticeably, and thereby restricting our possibilities for further experimentation. If time had been for it, we would have liked to learn more about the UV-degradation caused by natural sunlight. This would have required a more time than actually given at the beginning. Our understanding of IPBC degradation made by UV-light is necessary for this to be done, at the same time this type of experiment would require solitary use of the GC-MS. This said because some of the trouble we have faced might have been caused by other experiments that have been performed with known concentrations at about 0,5mg/ml and 1mg/ml. Natural samples to be collected could be very informative if collected over a year, to match the sunlight time calculated in other experiments (Lee et al.). We would have collected samples with small intervals, to determine when UV-degradation occurs, and at the same time collect samples from a covered basin. Hereby being capable of determining how much sunlight is causing decomposition of IPBC.

Adding another parameter to guard in the natural samples, could for instance be bacteria. When water is collected in nature from rain water, and in this case collected in a bucket after having run down the wood sample used for experiment, bacteria will be seen. These bacteria exist in water, and might have an important factor in degradation of IPBC. This factor though isn't fully determined and requires further experiments. So to compare IPBC degradation done by sunlight, and done in the dark, and at the same time following a third sample to compare how much degradation is done by bacteria, needs a lot of thought and planning. Background knowledge about bacteria and degradation performed by bacteria on other compounds will be needed to plan these well.

Expanding the thoughts on degradation, it could be interesting to see how much degradation is performed before water carries IPBC into water. Some degradation of the IPBC might already have happened when exposed to sunlight on the wood or have happened by bacteria attack, although IPBC is known to act well against bacteria and mould.

An idea for further investigation could be to look at the different groups on IPBC, and how the compound can be less toxic to mammals, but more toxic to fungi, algae or bacteria. The interest lies in the alkyl group that is found on IPBC, which has been seen to be toxic towards mammals, for instance in birds when they had eaten seed sprayed with an alkyl containing compound, which was used to protect the seed against fungal attacks. This alkyl group had then been replaced by a phenyl group, making it more effective against fungal attacks, but less toxic to mammals. Such investigations could be performed on IPBC to make it better in more senses, if further work was to be done with this compound.

The experiments performed in this work might be of some importance now, but less in the future. At presence IPBC is one of the most used preservatives, but another better chemical might replace IPBC, making research on IPBC of less importance. As well as limited use of wood in the future, trying to restore nature, might lead to other alternatives that might not need preservation.

Acknowledgment
We thank our supervisor John Mortensen (RU, Roskilde) for being available at all times and our external supervisors Iben Christensen (Teknologisk institut, Taastrup) and Emil Tange Engelund (Teknologisk institut, Taastrup) for their donation of IPBC and help. We would also like to thank Jakob Krake (RU, Roskilde) and Peter Christensen (RU, Roskilde) for helping us with the understanding of programming the HPLC and especially the GC-MS, which has been to great use in this project. A great thanks to Torben Lund (RU, Roskilde) for letting us use his laboratory.

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d. 21/05/08

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d. 07/05/08

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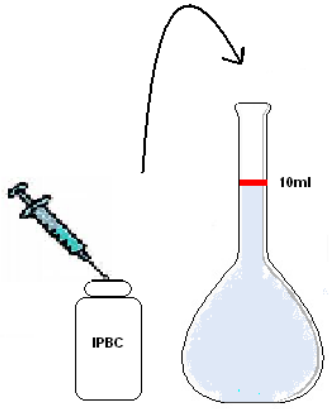
d. 07/05/08

Appendix

Solutions

a. The IPBC solution without naphthalene

1) IPBC solution of 100µg/ml (about).



IPBC needs to be diluted with dichloromethane to get a concentration about 100µg/ml. First, take 50µl of the pure IPBC solution with a syringe (50µl), and add into a volumetric flask (10ml). Then add dichloromethane up to 10ml.

2) IPBC solution of 50µg/ml (about).

Take 5ml fine solution from the first beaker and put it into a new volumetric flask (10ml), and add dichloromethane up to the 10ml line.

3) IPBC solution in 25µg/ml (about)

Take 5ml fine solution from the second beaker and put it into a new volumetric flask (10ml) volume, add dichloromethane up to the 10ml line.

4) To reach the 5µg /ml (5ppm) or smaller concentrations, the process has to be repeated 2 or 3 times.

b. The IPBC solution with naphthalene as an internal standard

1) The amount of naphthalene is 100µg/ml in larger concentrations of IPBC, and 10µg/ml in our samples with a lower concentration of IPBC. A solution with 50µg/ml naphthalene and 100µg/ml of IPBC is made.

First use a balance to get 1mg of pure naphthalene (crystals) and put it into a 10ml volumetric flask, and add dichloromethane up to the 10ml line, make sure to mix well. The concentration is now 100µg/ml. Then take 1ml of this solution into a new 10ml volumetric flask, and dilute it with dichloromethane to get a 10µg/ml solution.

Use a syringe (50µl) to move 50µl/ml of pure IPBC solution into a volumetric flask. Add the 10 µg/ml naphthalene-dichloromethane solution up to the standard marker. Now the solution of IPBC with naphthalene as an internal standard has been made.

2) In order to get smaller concentrations of the solution (IPBC and naphthalene), need to repeat the process in a2/a3.

c. Analysis of the IPBC solution

1) Sample for UV-Spectrum use

Use a pipette to move some of the prepared IPBC solution into a quartz cuvette. The sample is then placed in the UV-spectrophotometer ready for experiments.

2) Sample for GC-MS use

Make sure that the IPBC solution is mixed well, and then use a pipette to move some of the sample (about 2ml) into a GC-MS vial. The IPBC sample is then place in the GC-MS, and ready to be run.

Settings of the GC-MS

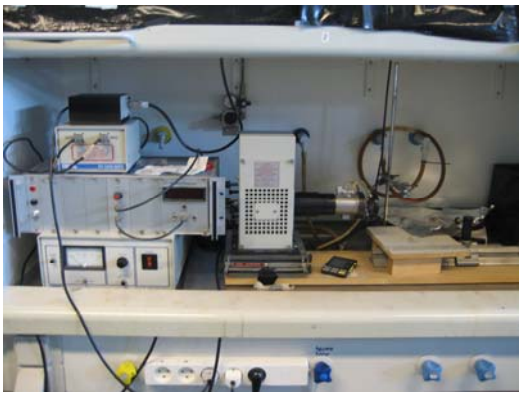
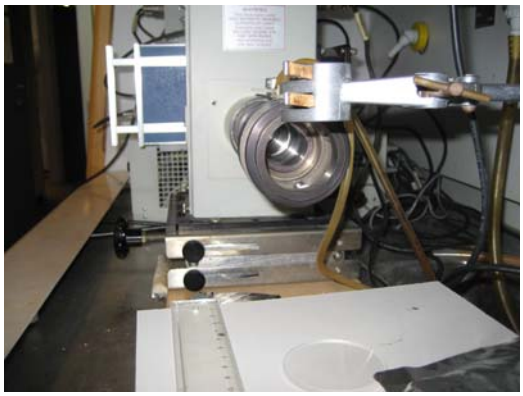
Through a lot of experiments with IPBC in dichloromethane at different concentrations, some changes were made to the method used as a standard in the GC-MS. These changes were done to optimize the readings of the machine at ppm levels (1ppm). A method we then chose to call IPBC6 had the following specificities:

- Split-less injector
- Sim mode (ions 112m/z and 281m/z)
- Pressure of He: 15.71Psi
- Flow of He: 1ml/min
- Injection in the back
- Injection volume: 5µl
- Inlet pressure: 13.1Psi
- Inlet flow: 34.7ml/min
- Inlet temperature: 280°C
- Column flow: 1µl (Column: 60m×250µm×0.3µm)
- Oven temperature: 35°C-300°C (rate of increase: 20°C/min)

This photo is of the GC-MS in the laboratory, and shows the GC-MS from the side.

UV-Degradation

When UV-light had to be replicated in the laboratory, a UV-lamp was used. The UV-lamp is a Hg lamp and has a broad spectrum, and is very intense. When a sample had been prepared, it was placed in a quartz cuvette 7cm away from the lamp, with the light centred onto the cuvette.

These two photos are taken from the laboratory, and show the UV-lamp from the side and from the front looking from where the cuvette would have been placed towards the lamp.

Natural sample - extraction

Solid-Phase Microextraction is used to extract IPBC from the natural sample containing rain water. The water is poured into a 50ml beaker, and only little at a time is put into a Solid-phase. The pressure is controlled, thereby making vacuum suck the water through the solid phase, which will extract the IPBC from the rest of the water. Methanol with naphthalene as an internal standard is used to wash out the IPBC from the solid phase. In the 2ml methanol sample that is used, 5µg/ml naphthalene is added. Knowing the concentration of naphthalene makes it possible to estimate the else unknown concentration of IPBC in the natural sample. The solution containing methanol, naphthalene and IPBC is then collected and transferred from a beaker to a vial, before running it in the GC-MS.