University of Bayreuth

Chair of Environmental Chemistry and Ecotoxicology

Source identification of trichloroacetic acid with preparative capillary gas chromatography and accelerator mass spectrometry

Dissertation

Faculty of Biology, Chemistry and Geosciences

University of Bayreuth

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Bayreuth, 2006

Die vorliegende Arbeit wurde im Zeitraum von August 2001 bis Februar 2005 im Arbeitskreis von Herrn Prof. Dr. Hartmut Frank am Lehrstuhl für Umweltchemie und Ökotoxikologie der Universität Bayreuth angefertigt.

Vollständiger Abdruck der von der Fakultät für Biologie, Chemie und Geowissenschaften der Universität Bayreuth zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften genehmigten Dissertation.

Einreicherung der Dissertation:	28.02.2006
Zulassung durch die Promotionskommission:	15.03.2006
Wissenschaftliches Kolloquium:	16.10.2006

Prof. Dr. H. Frank	1. Gutachter
Prof. Dr. C. Unverzagt	2. Gutachter
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Prof. Dr. E. Matzner	

For Paweł

Acknowledgements

Thanks are due to Prof. Dr. Hartmut Frank for guidance and stimulating discussions throughout the whole project.

For scientific discussions and a lot of interesting ideas I thank Dr. Radomir Čabala, Dr. Silke Gerstmann, and Dr. Michael Reissinger.

I thank Prof. Dr. Wolfgang Kretschmer, Andreas Scharf, and Dr. Thomas Uhl for their productive cooperation in realizing the project.

Financial support of the Federal Environmental Agency (UBA, Germany) is acknowledged.

For active support in the laboratory I thank Elke Bär, Agnes Bednorz, Manuela Hollmach, Joanna Pająk, and especially the co-worker who spent very long hours on preparing samples - Benjamin Schmidt.

The assistance of Dr. Radomir Čabala, Prof. Dr. Yafeng Guan, Dr. Xueliang Liu, Dr. Michael Reissinger, and Benjamin Schmidt in taking samples is greatly appreciated.

I also would like to express my gratitude to Jochen Beck, Dr. Eugen Christoph, Petra Gros, Dr. Ibrahem Hasan, Ingrid Hilpert, Huong Thu Thi Ngo, Dagmar Keis-Leichner, Franziska Knabe, Petra Kopeckă, Irmgard Lauterbach, Urte Lübcke-von-Varel, Katja Poxleitner, Dr. Elżbieta Przyk, Abed Abdel Qader, Dr. Cristina Schön-Velasco, Monika Suchocka, Wolfgang Weigl, Anna Weremiuk, Stefan Will, Joanna Znaleziona, and Manal el Zohary for providing a good working atmosphere, as well as Prof. Jacek Namieśnik and Ligia Zasławska from Gdańsk University of Technology for supporting.

Last but not least I appreciate the assistance of Brigitte Wittmann from the library, as well as that of the co-workers from the mechanical and glassblowing workshop.

Contents

List of abbreviations

AMS	Accelerator mass spectrometry
API/MS	Atmospheric pressure ionization/mass spectrometry
BP	Before present (set at 1950 AD)
CE	Capillary electrophoresis
DCC	*N,N'*-Dicyclohexylcarbodiimide
2,3-DCPA	2,3-Dichloropropionic acid
DDT	Dichloro-diphenyl-trichloroethane
DFA	2,4-Difluoroaniline
dw	Dry weight
EC10	Effect concentration (a point estimate of the toxicant concentration that would cause an observable adverse effect in 10 percent of the test organisms)
EPA	Environmental Protection Agency
ESI/FAIMS/MS	Electrospray ionization/high-field asymmetric-waveform-ion-mobility spectrometry/mass spectrometry
Et_2O	Diethyl ether
GC/ECD	Gas chromatography/electron capture detection
GC/EI/MS	Gas chromatography/electron-impact ionization/mass spectrometry
GC/FID	Gas chromatography/flame ionization detection
GC/IT/MS	Gas chromatography/ion-trap/mass spectrometry
GC/MS	Gas chromatography/mass spectrometry
GC/NCI/MS	Gas chromatography/negative-chemical ionization/mass spectrometry
IC	Ion chromatography

IC/ICP/MS	Ion chromatography/inductively-coupled plasma/mass spectrometry
i.d.	Inner diameter
LC/ESI/MS	Liquid chromatography/electrospray ionization/mass spectrometry
LC/ESI/MS/MS	Liquid chromatography/electrospray ionization/tandem mass spectrometry
LC/MS	Liquid chromatography/mass spectrometry
LS	Liquid scintillation
MIP	Molecularly imprinted polymer
MS	Mass spectrometry
MTBE	Methyl-*t*-butyl ether
Na-TCA	Sodium trichloroacetate
NCI	Negative-chemical ionization
NOEC	No-observed effect concentration
NOEL	No-observed effect level
o.d.	Outer diameter
PC-GC	Preparative capillary-gas chromatography
PDB	PeeDee Belemnite (limestone)
PEC	Predicted environmental concentration
PFBBr	Pentafluorobenzyl bromide
PFBHA	*o*-(2,3,4,5,6-Pentafluorobenzyl)-hydroxyamine
PFPDE	1-Pentafluorophenyl-diazoethane
PMC	Percent modern carbon
PNEC	Predicted no-effect concentration
PP	Polypropylene
REACH	Registration, Evaluation and Authorization of Chemicals

SIM	Selected-ion monitoring
SLMME/HPLC/UV	Supported-liquid-membrane micro-extraction/high-performance liquid chromatography/ultraviolet detection
SPE	Solid phase extraction
SPME	Solid phase micro-extraction
TBA-HSO_4	Tetrabutylammonium hydrogensulfate
TCA	Trichloroacetic acid, trichloroacetate
TCA-OMe	Trichloroacetic acid methyl ester
TIC	Total-ion current
TNO	Netherlands Organization for Applied Scientific Research
TPGC	Temperature-programmed gas chromatography
UV	Ultraviolet

1. Introduction

> *„…den Menschen und die Umwelt vor schädlichen Einwirkungen gefährlicher Stoffe und Zubereitungen zu schützen, insbesondere sie erkennbar zu machen, sie abzuwenden und ihrem Entstehen vorzubeugen.“*
>
> Gesetz zum Schutz vor gefährlichen Stoffen (Chemikaliengesetz - ChemG)

The still increasing numbers of chemicals produced and used as well as the amounts in which they are emitted or generally spoken introduced into the environment have required legal regulations in order to protect human health and the environment. The Chemical Act (Chemikaliengesetz) is the legal measure on the national level in Germany. Moreover, on the European level the new Registration, Evaluation and Authorization of Chemicals (REACH) system was authorized by the European Commission on 29 October 2003. It imposes on enterprises that manufacture or import more than one ton of a given substance annually the duty to register this substance in a central database. In addition, for chemicals which are produced in amounts higher than 100 tons annually an environmental risk assessment is necessary. The use of the most dangerous chemicals such as dichloro-diphenyl-trichloroethane (DDT) is restricted rigorously to certain applications. The REACH regulation has the aim of protecting human health and the environment while simultaneously maintaining the competitiveness and increasing the innovative capability of the chemical industry in the European Union.

During the risk assessment procedure for tetrachloroethene, which is the main precursor of trichloroacetic acid (TCA) a compound occurring in all environmental compartments, the question arose whether the reduction of its emission will result in decreased environmental concentrations of TCA. To answer this question it is essential to find out all possible sources of TCA. Numerous scientific analyses confirm its presence in the atmosphere, in soil, and in water. TCA concentrations in the atmosphere are in the lower ppq range (Klein, 1997; Peters, 2000), in rain water and soil in the ppb range (Frank, 1988; Hoekstra et al., 1999a; Klein, 1997; Reimann et al., 1996). However, mass balance studies performed with enormous uncertainties as reflected by the standard deviation of 80 % show that actual soil concentrations of TCA are higher than expected when taking all known anthropogenic contributions into account. Therefore, it is suggested that TCA can originate from both

anthropogenic and biogenic sources (Hoekstra et al., 1999a; Hoekstra et al., 1999b; Schöler et al., 2003b). Moreover, in some locations its concentrations in soil (predicted environmental concentration - PEC) exceed the predicted no-effect concentration (PNEC) value of 2.4 μg/kg. In consequence a PEC/PNEC ratio greater then one results indicating that TCA can cause toxic effects in this compartment (Ahlers et al., 2003). In such a case, risk reduction measures must be undertaken - in order to do this, the origin of TCA must be known.

From 1950 onwards, TCA has been used in agriculture as a herbicide because of its phytotoxicity. In 1995 its use was forbidden in Germany. It is tempting to ascribe the concentrations of TCA found nowadays to its former extensive use - however, this is highly unlikely as it is easily degraded in the environment. The half-life of TCA in soil is 14 - 90 days (Worthing et al., 1991), further considering its low Henry constant (7.4×10^4 mol/kg x atm) and high water solubility (1306 g/100 g H_2O) it seems implausible that TCA may have persisted in the pedosphere or biosphere for 10 years (Juuti et al., 1998).

To find out if it is possible to influence TCA-soil concentrations by reducing the production of its main precursor - tetrachloroethene - in the future, the question had to be answered: to which extent is TCA formed from anthropogenic precursors and introduced into soil and to which one is it formed from biological precursors in soil.

2. Objectives of the thesis

The aim of the present thesis is to develop and apply a method for radiocarbon source identification of environmental chemicals which are present at trace levels in soil, using TCA as an exemplary compound.

The differentiation between the biogenic and anthropogenic origin of TCA is achieved by determining the $^{14}C/^{12}C$ ratio. This determination is based on the fact, that materials derived from assimilation of atmospheric carbon dioxide contain the typical equilibrium concentration of ^{14}C ($^{14}C/^{12}C = 1.2 \times 10^{-12}$), whereas fossil materials, which are several hundred million years old, do not contain ^{14}C due to its relatively short half life of 5730 years. The $^{14}C/^{12}C$ ratio of TCA isolated from soil samples is determined with accelerator mass spectrometry (AMS) which is sensitive enough for isotope ratio measurement in the range of 10^{-12} to 10^{-15} (Kretschmer, 2000). At the beginning of the project, the AMS facility, situated in Erlangen, was equipped with a graphite ion source requiring a minimum of 30 μg of carbon for the analysis. Consequently, a suitable sampling site burdened with high amounts of TCA had to be found which allowed to obtain the required carbon amount. Recently, the original ion source has been replaced by a gas ion source allowing to perform the analysis with smaller amounts of carbon such reducing the original needed 30 μg of carbon to 1 - 5 μg.

In order to obtain the required amount of carbon, a soil sample must be taken and the analyte of interest isolated with a sequential extraction during which fatty acids, waxes, lipids etc. are removed. The thus purified sample must be separated applying preparative capillary-gas chromatography (PC-GC) which represents a practical method for the isolation of individual compounds from complex organic matrices (Eglinton et al., 1996). So obtained pure TCA must be combusted to carbon dioxide and subsequently fed into the ion source of an AMS facility. Radiocarbon measurements allow to determine the origin - anthropogenic or biogenic - of TCA or any environmental chemical.

3. TCA in the environment

3.1 Application and sources of TCA

Industrially TCA is produced by the chlorination of acetic acid. The crude product, containing about 95 % TCA, is isolated by crystallization. Thereby, the purity is increased through centrifugation or re-crystallization steps (Koenig et al., 1986). Only some data are available about TCA production in Germany; in 1993, about 1000 t were produced; 670 t for export, 250 t for the production of sodium trichloroacetate, 50 t for the production of trichloroacetic acid ethyl ester and 30 t for sale (mainly to producers of pharmaceuticals). During production and processing, discharges into waste water are reported to amount to approximately 1 kg/a, and via exhaust air to about 17 kg/a. As roughly 100 t of sodium trichloroacetate (Na-TCA) are used in Germany in the formulation of textile finishing products, 2 t/a are released to waste water treatment plants (BUA-report, 1995).

Na-TCA was used as herbicide which exhibits a low specific mechanism of action. It is applied directly into the soil usually as water soluble powder (Domsch, 1992). However, since 1989, its sale and import as a herbicide is prohibited in Germany and Switzerland. Nowadays, TCA is used as an etching or pickling agent in the surface treatment of metals, as a swelling agent and solvent in the plastic industry, as an albumin precipitating agent in medicine, as an auxiliary in textile finishing, as an additive to improve high-pressure properties in mineral lubricating oils, and as a glue precipitant. In the manufacturing of photographic films, TCA is used as an etching agent. It is also widely used in dermatology to remove warts and hard skin, and to treat various skin afflictions. TCA esters are important starting materials in organic syntheses (Koenig et al., 1986; OECD, 2000).

Furthermore, TCA is formed as a by-product (OECD, 2000):

- during the atmospheric oxidation of C_2-chlorocarbons which is the most significant process in regard to the release of TCA into the environment;
- during the chlorination of drinking water and swimming pools;
- in chemical cleaning units using tetrachloroethene;
- in electroplating facilities treating cyanide containing waste water with NaOCl;
- in textile washing facilities using NaOCl as bleaching or disinfection agent;

- during the bleaching of paper with agents containing chlorine in paper mills (Juuti et al., 1995).

Although there are no direct emissions of TCA in the atmosphere during its production or use, the substance is formed in this compartment due to the photooxidation of tetrachloroethene and 1,1,1-trichloroethane, respectively. These atmospheric processes are considered to be the main sources of TCA in the environment.

As chloroform was found at high levels in the soil-air (higher than in the atmosphere) of a site where forest decline is most severe and this compound is unlikely to exhibit phytotoxicity, it was suggested that it arises from decarboxylation of TCA. Moreover, similar fluctuations of chloroform and tetrachloroethene levels were found indicating that the former is a product of the latter formed via decarboxylation of TCA which is a product of atmospheric oxidation of tetrachloroethene (Frank et al., 1989a; Frank et al. 1990a; Frank et al., 1991c).

The ubiquitous occurrence of C_2-halocarbons in conjunction with efficient photooxidation in air may explain the formation of relatively large amounts of TCA in rural and mountain regions (Frank et al., 1990a). So, TCA may be regarded as an indicator for the distribution and deposition of secondary air pollutants arising from atmospheric oxidation of C_2-halocarbons (Frank et al., 1994; Gullvag at al., 1996).

3.1.1 Formation of TCA from tetrachloroethene

Tetrachloroethene is mainly used as solvent in dry cleaning, metal degreasing, and extraction processes. Its European production volume was 164,000 t/a in 1994, its world production 553,000 t/a in 1984. Tetrachloroethene is ubiquitous in the atmosphere, however, with a strong concentration gradient from the emission areas to the global background pollution. Typical concentrations for rural areas are 0.1 to 0.5 $\mu g/m^3$, and for cities 0.5 to 15 $\mu g/m^3$ (OECD, 2000).

In the troposphere, tetrachloroethene reacts both with OH-radicals and with Cl-radicals, the latter reaction representing the main TCA source. About 87 % of tetrachloroethene react with OH-radicals in the atmosphere and the main, if not the only, product of this reaction is phosgene which is unlikely to form TCA or its precursors. However, in the reaction between tetrachloroethene and Cl-radicals the pentachloroethoxy radical is formed which, in turn, yields phosgene (15 %) and trichloroacetyl chloride (85 %). When atmospheric trichloroacetyl chloride is dissolved

in cloud water and hydrolyzed it forms TCA in yields of 46 %. It has been calculated that overall approximately 5 % of tetrachloroethene released into the atmosphere are converted into TCA (McCulloch, 2002).

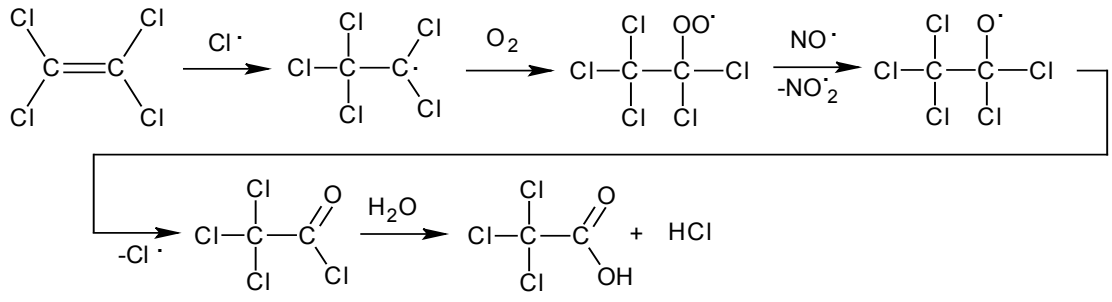

Fig. 1: Formation of TCA from tetrachloroethene in the atmosphere.

3.1.2 Formation of TCA from 1,1,1-trichloroethane

1,1,1-Trichloroethane is mainly used as solvent in industrial processes and for degreasing of metallic surfaces. Its global production is reported to have been 678,000 and 726,000 t/a in 1988 and 1990, respectively. 1,1,1-Trichloroethane causes depletion of the stratospheric ozone. Therefore, its production for emissive uses in Europe was phased out under the Montreal Protocol at the end of 1995. Due to its long lifetime (3.8 to 6 years, Chang et al., 1977) 1,1,1-trichloroethane is ubiquitous in the atmosphere at a concentration range of 0.5 to 2 $\mu g/m^3$ (OECD, 2000).

The principal product of its tropospheric decomposition is chloral, the reaction starting with hydrogen abstraction by an OH-radical. Of the total amount of 1,1,1-trichloroethane released into the atmosphere 84 % form chloral. The rest of it is either photolyzed in the stratosphere and forms acetyl chloride, or hydrolyzed in the oceans where it forms acetic acid and 1,1-dichloroethene (Euro Chlor, 2001).

There are three possible sinks for tropospheric chloral: a) predominantly photolysis, with 4 to 36 hours lifetime depending on the quantum yield assumed (Barry et al., 1994; Rattigan et al., 1998; Jordan et al., 1999); b) reaction with OH-radicals with a lifetime of 5 to 10 days (Rattigan et al., 1998; Jordan et al., 1999), and c) dissolution in cloud water. Although chloral has a high water solubility, its uptake in clouds is governed by atmospheric mixing; the lifetime of this process is about 10 days. The fraction of chloral absorbed on clouds is calculated to be 1.3 % of the total

amount of 1,1,1-trichloroethane released. Further oxidation of the dissolved chloral hydrate takes place in clouds by OH-radical, such the formation of TCA is possible (Frank et al. 1991b; Euro Chlor, 2001). TCA formation through 1,1,1-trichloroethane oxidation in the gas phase is governed by atmospheric OH-radical and HO_2-radical concentrations as well as by the NO_X/HO_2 ratio (Folberth et al., 2003; Hoekstra, 2003).

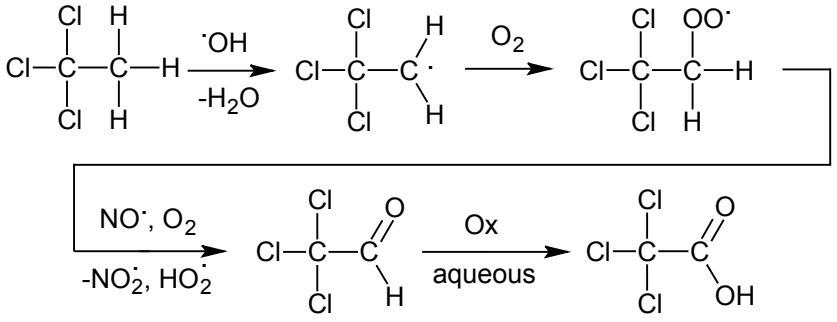

Fig. 2: Formation of TCA from 1,1,1-trichloroethane in the atmosphere.

3.1.3 Suggested natural formation of TCA

Although TCA seems to originate mainly from the atmospheric degradation of tetrachloroethene and 1,1,1-trichloroethane, the mass balance calculations giving a rough estimation (uncertainties of about ± 80 %) of its fluxes in the environment indicate that there could be an additional biogenic source of TCA in soil which means that TCA could be formed from naturally occurring compounds as a result of naturally occurring processes (Hoekstra et al., 1999a; Schöler et al., 2003b). This is still a non-proven suggestion and only one biotic pathway via peroxidases may perhaps be considered for the natural formation of TCA. The chloroperoxidase of the fungus *Caldariomyces fumago* is able to produce reactive chlorine species (hypochlorous acid) and its occurrence is ubiquitous in organisms and plants. In the presence of hydrogen peroxide in an optimum pH range between 3.0 and 3.5 a chloroperoxidase-like activity was observed (Asplund et al., 1993; Schöler et al., 2003a). TCA is suggested to be formed in-situ from aliphatic acids like acetic acid and malic acid or humic acids in chloroperoxidase catalyzed processes (Haiber et al., 1996; Hoekstra et al., 1999b).

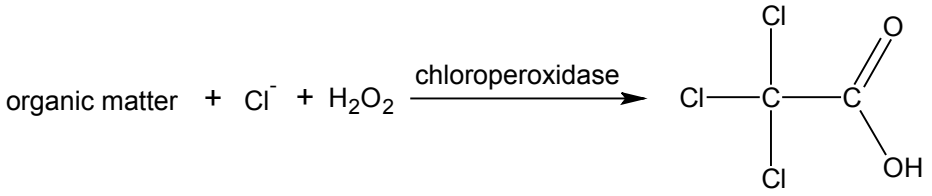

Fig. 3: Biotic formation of TCA.

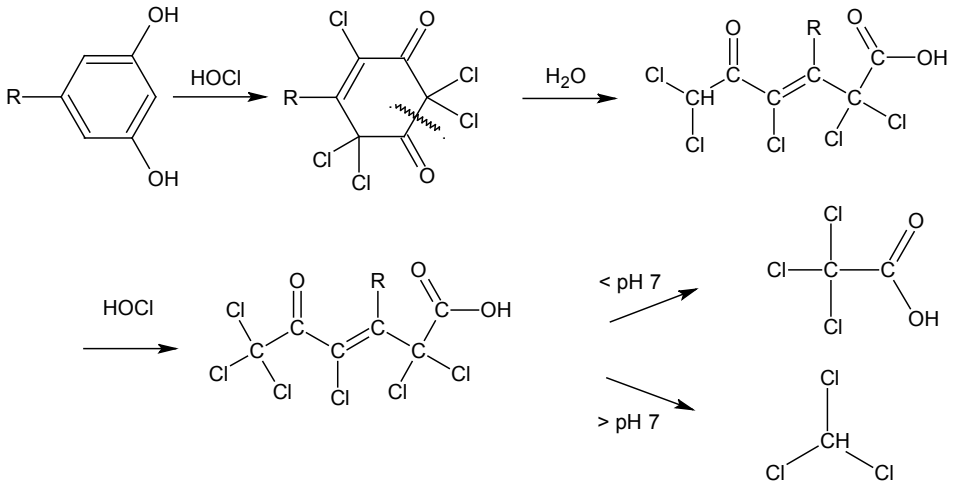

Fig. 4: Speculative formation of TCA and chloroform from resorcinolic substances (Hoekstra et al., 1999b).

The abiotic formation of TCA from soil and humic acids was also investigated. A multitude of phenolic substances which are common structural components of natural organic matter can be transformed into TCA. The concentration of formed TCA increases with increasing amounts of H_2O_2 and Fe^{3+}. Exposing the reaction mixture of humic acids and H_2O_2 to ultraviolet (UV) light also enhances the production of TCA. It is supposed that Fe^{3+} is reduced to Fe^{2+} by redox-sensitive organic material and that probably a Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$) takes place. Thus hydroxyl radicals are generated which are able to oxidize chloride (when present) to elemental chlorine and lead to non-specific chlorination of organic compounds. The C-Cl bond is probably formed in a radical-related mechanism. This way of TCA formation is suggested to be important since humus, iron, and chloride are widespread in soil (Fahimi et al., 2003). An alternative explanation for the effect of Fe^{3+} could be the formation of a humic acid-Fe^{3+}-complex

that acts similar to a heme group in the chloroperoxidase-mediated reaction leading to the formation of hypochlorous acid (Hoekstra, 2003).

TCA can also be produced from the biotransformation of trichloroethene by methane utilizing bacterium *Methylocystis* sp. strain M in neutral pH conditions. Trichloroethene is initially converted to chloral by soluble methane monooxygenase and then oxidized to TCA (Saeki et al., 1999).

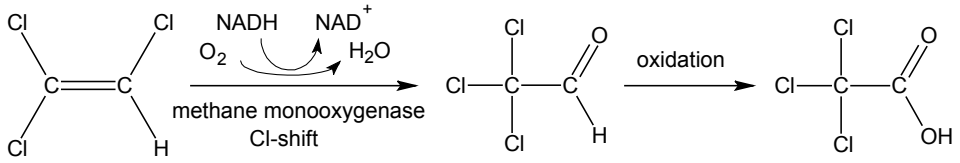

Fig. 5: Hypothetical pathways of trichloroethene degradation by Methylocystis sp. (Saeki et al., 1999).

It is also speculated that natural TCA formation is supported by the fact that this compound was prevalent (7 - 10 ng/L) in firn representing the past 100 years of snow accumulation in Antarctica and glacier ice (5 - 13 ng/L); the samples were taken from permanently frozen ice layers with well preserved primary structures and no visible crevasses (von Sydow et al., 1999). However, the determined TCA concentration values do not differ considerably from the blank values which are lower than 10 ng/L and from the publication it is not clear whether the values of TCA concentration were corrected for the blanks or not.

3.2 Environmental concentrations of TCA

TCA can be found in different environmental compartments, mainly the atmosphere, hydrosphere (rain, fog, surface water), biosphere (leaves, needles), and pedosphere (different soil horizons).

Once TCA is formed in the free atmosphere it is, due to its low Henry constant, likely to be taken up into atmospheric aerosols which can either be dry-deposited on leaves and needles of trees and conifers or directly washed out by rain; from these compartments TCA is transported into soil either as canopy run-off or as direct wet deposition. In soil it is very mobile and migrates into ground water, it can also

undergo bacterial and fungal degradation. TCA is mainly translocated from soil to the leaves/needles of a plant in its transpiration stream via the roots. Older needles always exhibit higher TCA levels than younger ones indicating a tendency of conifers to accumulate TCA in the plant foliage (Norokorpi et al., 1993). Moreover, a seasonal variation in TCA concentration was observed during the course of a year. TCA levels increase continuously in summer and autumn as in a hot and dry period the water and TCA uptake from soil is high, and start to decrease in early winter (Frank, 1988; Frank et al., 1990c; Frank et al., 1992). A correlation between the degree of trees defoliation and the TCA concentration was also found (Frank et al., 1992; Frank et al., 1994; Norokorpi et al., 1995).

Usually, TCA occurs in the environment as trichloroacetate, and is easily decarboxylated. However, as the pH in forest soil is generally in the range of 3.5 - 5.0 TCA is rather stable against decarboxylation in this compartment. The presence of light increases its degradation rate but only in the very top soil layer (Hoekstra, 2003). Some environmental TCA concentrations are given in table 1.

Environmental compartment	**Concentrations**
Soil (Frank, 1988; Hoekstra et al., 1999a)	0.2 - 380 [ng/g]
Conifers (Juuti et al., 1995; Juuti et al., 1996)	1 - 276 [ng/g]
Air (Klein, 1997; Peters, 2000)	6 - 700 [pg/m^3]
Air particulate matter (Bakeas et al., 2003; Heal et al., 2003)	0.9 - 184 [pg/m^3]
Fog (Römpp et al., 2001)	0.02 - 2.0 [μg/L]
Rain water (Reimann et al., 1996; Klein, 1997)	0.005 - 0.71 [μg/L]
Snow (von Sydow et al., 1999; von Sydow et al., 2000)	0.022 - 0.348 [μg/L]
Rivers (Klein, 1997; Hashimoto et al., 1998a)	0.003 - 22.0 [μg/L]
Lakes (Müller at al., 1996; Scott et al., 1999)	0.06 - 0.53 [μg/L]
Drinking water (Berg et al., 2000; Villanueva et al., 2003)	0.02 - 25.5 [μg/L]

Tab. 1: TCA concentrations in environmental compartments.

3.3 Physico-chemical properties of TCA

TCA a polar and hydrophilic substance forms white deliquescent crystals and has a characteristic odor. Its esters and amides are readily formed. TCA decomposes to chloroform and carbon dioxide when its aqueous solution is exposed to heat. The decomposition is particularly fast in the presence of organic or inorganic bases. Besides water, aniline, resorcinol, and activated carbon catalyze the decomposition. Purely thermal decomposition takes only place when the boiling point is exceeded resulting in the formation of chlorinated hydrocarbons, carbon monoxide and dioxide, and phosgene (Morris et al., 1991; Koenig et al., 1986). Due to its pKa value, TCA is present as trichloroacetate in environmental conditions. As it is completely ionized and has a low Henry constant, it cannot be transported from the water compartment into the atmosphere. The low octanol/water partition coefficient neither allows TCA to bio-concentrate in food chains nor to adsorb on soil colloids. Its physico-chemical properties are given in table 2.

Property	Trichloroacetic acid
CAS Nr.	76-03-9
IUPAC name	2,2,2-trichloroethanoic acid
Molecular formula	$C_2HCl_3O_2$
Molar mass	163.39 g/mol
Physical state	solid
Melting point (Morris et al., 1991)	59 °C
Boiling point (Morris et al., 1991)	197.5 °C
Density at 64 °C (Morris et al., 1991)	18
Dissociation constant (pK_a) at 25 °C (Bowden et al., 1998)	0.26
Distribution coefficient octanol/water (log K_{ow}) (OECD, 2000)	-0.27
Vapor pressure at 20 °C (GESTIS-Stoffdatenbank)	10 Pa
Water solubility at 25 °C (Morris et al., 1991)	1306 g/100 g H_2O
Henry constant at 25 °C (Bowden et al., 1998)	$7.4x10^4$ mol/kg×atm

Tab. 2: Physico-chemical properties of TCA.

3.4 Toxicity of TCA

3.4.1 Ecotoxicology

TCA was used as a pre-emergence herbicide for the control of many annual and perennial grasses in crop and non-crop fields because of its phytotoxic effect. It causes formative effects and growth inhibition, induces chlorosis (lack of green pigments) and necrosis (the death of some or all cells in an organ or tissue) of light-exposed leaves, accelerates the degradation and eventually death of conifers, such representing one of the atmogenic stress factors causing forest decline. A main phytopathogenic effect of TCA is its interference with meristematic activity. Furthermore, it can reduce the formation of the wax layer of cuticules and such increases the water permeability. The inhibition of the pantothenate synthesis, the precursor of coenzyme A, has been suggested to be the main cause of its phytotoxicity (Frank et al., 1990b; Frank, 1991a; Gullvag et al., 1996). Moreover, it was proven that the uptake route of TCA into the needles of Scots pine leads via roots and needle surface layers. Primary, its translocation from soil into the plant occurs via the transpiration stream. First, TCA is accumulated in the roots, stem, and branch cells, then it reaches the needles along the concentration gradient. Since TCA is hydrophilic, its uptake via the needle surface is of lesser importance than that via the roots (Frank, 1988; Sutinen et al., 1995; Sutinen et al., 1997).

TCA is classified as “non-biodegradable” with “low bio-accumulation potential” for fish and “high bio-accumulation potential” for terrestrial plants causing forest decline. The most sensitive environmental species to TCA is the algae *Chlorella pyrenoidosa* (14d-NOEC = 0.01 mg Na-TCA/L = 8.6 μg TCA/L) followed by pine species such as *Pinus sylvestris* (60d-EC10 = 0.12 mg Na-TCA/kg). Applying a safety factor of F = 50, the PNEC for the aquatic compartment has a value of 0.17 μg/L, and that for soil one of 2.4 μg/kg dry weight (dw). The environmental hazard assessment shows that TCA represents a risk for the hydrosphere and especially for the soil compartment (OECD, 2000). In recent studies it is concluded that current environmental concentrations should not have a direct impact on aquatic organisms, e.g. acute mortality of fish or zooplankton (Hanson et al., 2004).

3.4.2 Mammalian toxicity

The herbicide Na-TCA causes in humans acute irritations of mucous membranes and respiratory difficulties when applied as aerosol. Further acute exposure effects which can occur are mild to moderate dermal and ocular irritation. However, TCA is not readily absorbed through the skin.

The toxicity of TCA to humans was estimated based on acute toxicity tests mainly conducted with mice and rats by administrating Na-TCA as single oral, dermal, and inhalational dose, respectively. As a result TCA can be designated as non-toxic. It is corrosive to rabbit skin and strongly irritant to their eyes but it does not cause sensitization. The sodium salt has a significantly lower irritation potential than the acid and is used in 50 % concentrations as cauterizing agent. In different experiments with rats the NOEL was determined to be 4000 ppm (356 mg Na-TCA/kg body weight/day) when administrated for 4 months or 1600 ppm (approx. 80 mg Na-TCA/kg body weight/day) when given for 2 years. In a sub-chronic feeding study with dogs lasting for 90 days the NOEL was found to be 500 ppm (approx. 30 mg Na-TCA/kg body weight/day).

TCA carcinogenicity studies were negative; however, it causes liver toxicity in rats as shown in a drinking water study administering concentrations from 0 to 500 mg TCA/L and lasting for 100 - 104 weeks. Mechanistic investigations showed that TCA induces peroxisome proliferation in rodent hepatocyte cultures but not in human liver-cell cultures. A tumor-initiating action in humans is unlikely for TCA (OECD, 2000). The primary source of exposure to TCA for humans is drinking water wherein TCA occurs at the ppb level as a chlorination by-product (Berg et al., 2000; Villanueva et al., 2003). Since the early 1970's, numerous epidemiological studies have attempted to assess the relationship between chlorination by-products in drinking water and several human cancers. None of these studies could show an association of exposure to TCA with an increased incidence of cancer (IRIS, 1996).

A reproductive toxicity study shows TCA to be a developmental toxin in rats at doses $\geq$ 330 mg/kg body weight exhibiting a percentage of 9 % of malformations of soft tissue, especially in the cardiovascular system (OECD, 2000).

Although Na-TCA increases the chromosome aberration frequency in the culture of human peripheral lymphocytes, the available evidence does not suggest that TCA is mutagenic which is consistent with the view that liver tumors observed in mice

treated with TCA arose by a non-genotoxic mechanism (peroxisome proliferation) (OECD, 2000).

3.5 Analytical methods for haloacetate analysis in the environment

For the determination of haloacetates in environmental samples, a large number of analytical methods was developed but not all of them can be applied in routine analysis due to their insufficient sensitivity. Most of the methods require conversion of the respective haloacetate into a more volatile and less polar ester. Although the derivatization process is time- and work-consuming and some substrates are toxic and carcinogenic, only this approach can ensure sufficient sensitivity for analysis.

3.5.1 Gas chromatography/electron capture detection (GC/ECD)

There are only two official methods used for the determination of the concentration of haloacetates in drinking water: the Environmental Protection Agency (EPA) methods number 552.0 and 552.2 which include liquid-liquid extraction, derivatizaton with diazomethane and determination of the so-formed methyl esters with GC/ECD. The limit of detection for trichloroacetic acid methyl ester (TCA-OMe) is 0.08 μg/L (Hodgeson et al., 1990; Munch et al., 1995).

After percolating the water samples over an ion-exchange resin, trapping and eluting them with methanol solution acidified with sulfuric acid, while simultaneously esterifying them, and then extracting them with cyclohexane, it was possible to reach a detection limit of 0.07 μg/L for TCA with GC/ECD (Benanou et al., 1998). This method is used for the determination of haloacetates in drinking water.

When using *o*-(2,3,4,5,6-pentafluorobenzyl)-hydroxyamine (PFBHA)-acidic methanol esterification of haloacetates, detection limits under 1 μg/L can be reached (Xie et al., 1998).

It is also possible to determine the concentration of TCA in water samples by GC/ECD without derivatization. In this case, TCA is thermally decarboxylated to chloroform which is then injected on the column (Frank et al., 1990c). Thereby, a limit of detection of 0.1 μg/L for TCA can be obtained (Drechsel et al., 2001).

Overall low detection limits make GC/ECD a useful method for the determination of haloacetates but the drawbacks of this method are long sample preparation and analysis time.

3.5.2 Gas chromatography/mass spectrometry (GC/MS)

To determine TCA applying GC/MS with negative-chemical ionization (NCI) it is converted into a volatile ester with 1-pentafluorophenyl-diazoethane (PFPDE) such gaining sensitivity 100 times higher than that of any given TCA alkyl ester. While the detection limit of the methyl ester of TCA is 300 ng/L, that of the pentafluorophenyl-ethyl ester is 3 ng/L (Renschen, 1995). In comparison to other haloacetate derivatives the sensitivity can increase even 2800 times. This is the most sensitive method used for TCA analysis. Therefore, this method is also applied in the present thesis for TCA quantification.

The methyl esters of haloacetates obtained after derivatization with diazomethane can also be determined by mass spectrometry with positive ionization. In this regard, solid phase micro-extraction (SPME) is used as an alternative for liquid-liquid extraction (see EPA-methods). Using a haloacetate methyl ester, headspace sampling applying SPME, and gas chromatography/ion-trap/mass spectrometry (GC/IT/MS) a limit of detection of 0.02 μg/L (Sarrion et al., 2000) or 0.01 μg/L (Sarrion et al., 1999) for TCA can be reached.

Other possible derivatization reagents for TCA when applying GC/MS are 2,4-difluoroaniline and acidic methanol. In the first case, the detection limit of TCA is 0.2 μg/L (Saeki et al., 1999), in the latter case, the method performance is comparable to that of EPA method 552.2 achieving a detection limit of 0.15 μg/L (Xie, 2001). However, it offers advantages like fewer interfering peaks, smoother baselines, and comparatively shorter analysis times.

The GC/MS method is very sensitive and selective but, as GC/ECD, it requires long sample preparation and analysis time.

3.5.3 Capillary electrophoresis (CE)

For the determination of the concentration of haloacetates in drinking water also solid phase extraction (SPE) followed by CE and UV-detection can be used.

Although the detection limit of 2 μg/L for TCA is higher than those reached with GC/ECD or GC/MS, and the analytes have to be enriched prior to the analysis, unlike GC, this method requires no derivatization step, so the overall analysis time can be shortened considerably (Martinez et al., 1998).

When CE is combined with atmospheric pressure ionization/mass spectrometry (API/MS) the derivatization step can also be omitted. The sample pre-treatment comprises liquid-liquid extraction using methyl-*t*-butyl ether (MTBE), drying (nitrogen stream), and dissolution in methanol. A detection limit of 0.1 mg/L can be obtained for TCA (Ahrer et al., 1999).

The advantage of CE is that it requires relatively short sample preparation time, however, the detection limits make it appropriate only for screening.

3.5.4 Liquid chromatography/mass spectrometry (LC/MS)

Another method used to determine the concentration of haloacetates in drinking water is liquid chromatography/electrospray ionization/mass spectrometry (LC/ESI/MS). Ion pairing reagents such as di-n-butylamine, N,N-dimethyl-n-butylamine, and tri-n-butylamine are employed. Di-n-butylamine ensures the best chromatographic resolution and results in a limit of detection of 83 ng/L for TCA. The sample preparation involves only filtration and addition of the ion-pairing reagent (Takino et al., 2000). Using liquid chromatography/electrospray ionization/tandem mass spectrometry (LC/ESI/MS/MS) a detection limit of 75 ng/L for TCA can be obtained (Gros, 2002). The sensitivity of the latter can be improved to 0.9 μg/L when using triethylamine as volatile ion-pairing agent and adding a SPE step during sample preparation (Loos et al., 2001). This approach can also be used to determine TCA in human urine reaching a detection limit of 0.5 μg/L (Kuklenyik et al., 2002).

Another variation in sample preparation comprises acidification (pH < 0.5) and extraction with MTBE of a given water sample. In this way a detection limit of 0.07 μg/L for TCA can be obtained when using LC/ESI/MS/MS (Hashimoto et al., 1998b).

A combination of electrospray ionization, high-field asymmetric-waveform-ion-mobility spectrometry, and mass spectrometry (ESI/FAIMS/MS) allows to achieve a detection limit of 36 ng/L for TCA (Ells et al., 2000) or 180 ng/L (Gabryelski et al.,

2003). An advantage of this method is a significant reduction in the chemical background from ESI as selected ions can be filtered from a complex mixture.

Although LC-MS is a very fast and sensitive method, the matrix interferences are very strong.

3.5.5 Supported-liquid-membrane micro-extraction/high-performance liquid chromatography/UV detection (SLMME/HPLC/UV)

Recently, the SLMME method has been used for water analysis. TCA is extracted into a supported-liquid-membrane, and then back-extracted into an acceptor solution which can be analyzed directly by HPLC/UV. The extraction procedure takes one hour, and the chromatographic separation 15 minutes. The method is simple, sensitive, selective, and does not require a derivatization step. A sample can be enriched 300 to 3000-fold. The detection limit of 0.05 μg/L makes the method an attractive alternative to the US EPA methods (Kou et al., 2004).

3.5.6 Ion chromatography (IC)

TCA in drinking water is also determined by ion chromatography with suppressed electrical conductivity detection. Employing an anion-exchanger for the separation of analytes the limit of detection for TCA is 0.85 μg/L (Nair et al., 1994; Liu et al., 2003; Liu et al., 2004a).

Vacancy ion-exclusion chromatography is another method for the determination of the concentration of haloacetates in water. For this purpose, a weakly acidic cation-exchange resin in the H^+ form and conductivity detection are used. The separation is mainly based on a combination of ion-exclusion and adsorption effects resulting in a detection limit of 25 μg/L for TCA. The sample solution is used as a mobile phase and pure water as the injected sample (Helaleh et al., 2003). Applying SPE prior to the microbore ion chromatography, a detection limit of 1.1 μg/L can be obtained for TCA (Barron et al., 2004).

When using ion chromatography/inductively-coupled plasma/mass spectrometry (IC/ICP/MS), the detection limit for TCA is 24 μg/L. With an anion-exchange column functionalized with very hydrophilic quaternary ammonium groups, the analysis can be performed in 15 minutes (Liu et al., 2004b).

IC is a moderately sensitive method and shows a matrix dependence similar to LC-MS but its analysis time is longer.

3.5.7 Sensor based on molecularly imprinted polymer (MIP) membrane

Recently, a conductometric sensor based on MIP was developed for the fast and sensitive screening of complex mixtures of haloacetates in drinking water. The detection is achieved by TCA-imprinted polymers synthesized through the copolymerization of 4-vinylpyridine and ethylene glycol dimethacrylate in the presence of a TCA template in acetonitrile by a multi-step swelling polymerization method. These polymers can bind selectively to the template molecule and haloacetate derivatives. Hereby, the limit of detection for TCA is 1 μg/L (Suedee et al., 2004).

3.6 Derivatization methods

In general, the application of GC for the determination of TCA requires a derivatization step to enhance the sensitivity of the analytical method. In the following, a short overview is given about possible derivatization reagents and their advantages and disadvantages are discussed.

3.6.1 1-Pentafluorophenyl-diazoethane (PFPDE)

Haloacetates are derivatized with PFPDE in etheral solutions by keeping the reaction mixture at room temperature for about 12 hours and then analyzed with gas chromatography/negative-chemical ionization/mass spectrometry (GC/NCI/MS). One disadvantage is that PFPDE has to be synthesized as it is not commercially available. However, the 100-fold better sensitivity for the TCA pentafluorophenyl-ethyl ester as compared to the methyl ester makes this method very attractive and the most sensitive one (Renschen, 1995).

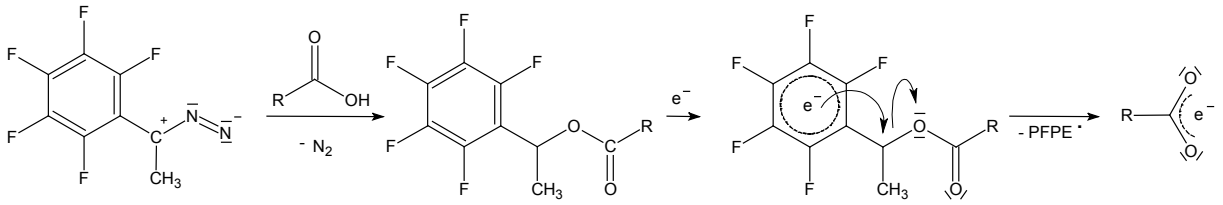

Fig. 6: Derivatization of haloacetates with PFPDE and fragmentation in GC/NCI/MS (Hofmann et al., 1990). R = X_3C, X_2HC, XH_2C; X = Cl, Br, I.

3.6.2 2,4-Difluoroaniline (DFA)

A further derivatization reagent used for chloroacetic acids is DFA; the reaction is catalyzed by *N,N'*-dicyclohexylcarbodiimide (DCC). In this way, the TCA difluoroanilide is synthesized and a detection limit of 0.6 μg/L for TCA is obtained with GC/ECD (Ozawa et al., 1987; Ozawa et al., 1990).

3.6.3 Acidified methanol

On-column methylation of haloacetates trapped on an anion-exchange resin with acidified methanol is another possible derivatization method although not commonly used. Time-intensive sample preparation combined with low precision and low accuracy of the GC/ECD method are the reasons. However, by using GC/MS instead of GC/ECD the analysis time can be reduced while at the same time an increased sensitivity is gained (Benanou et al., 1998; Urbansky, 2000; Xie, 2001).

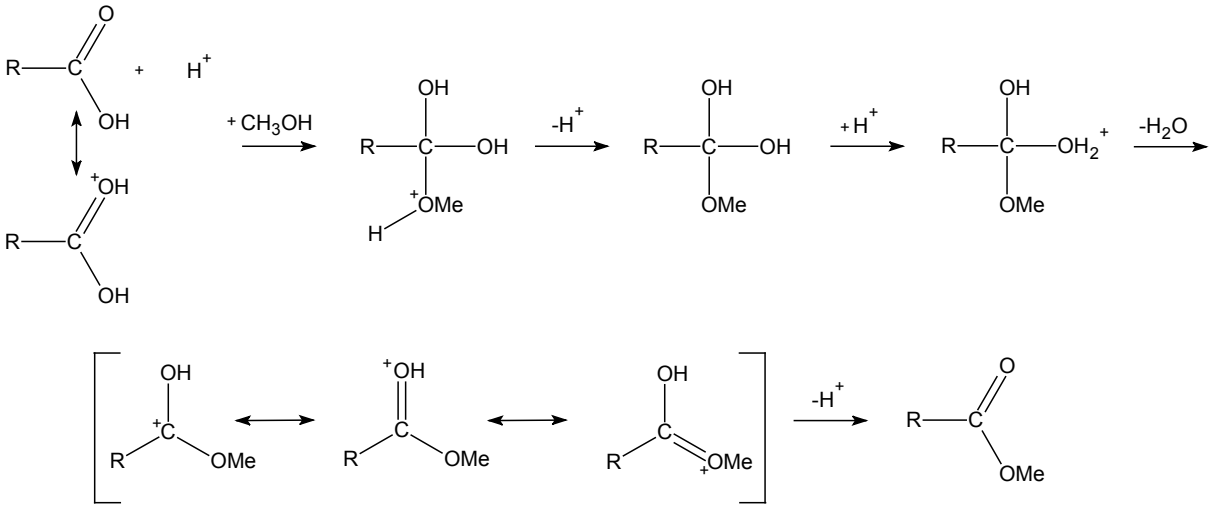

Fig. 7: Derivatization of haloacetates with acidic methanol (Urbansky, 2000). R = X_3C, X_2HC, XH_2C; X = Cl, Br, I.

3.6.4 Diazomethane

When using diazomethane as derivatizing reagent for TCA, the following points have to be taken into account. First, diazomethane is a carcinogen. Second, it is a mild explosive and therefore it cannot be stored for longer time. As a consequence, it has to be prepared freshly every time. However, the reaction time is short, diazomethane is formed in high yields and side reactions are minimal. To this end, methanolic KOH solution is dropped into N-methyl-N-nitroso-4-toluenosulfonamide. The so-formed diazomethane is passed through a solution of TCA which methyl ester is then formed. TCA-OMe can be determined with GC/ECD, GC/MS or GC-FID (Blau et al., 1993).

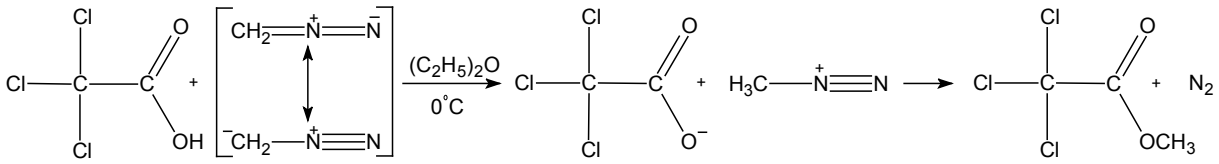

Fig. 8: Derivatization of trichloroacetate with diazomethane.

3.6.5 Dimethylsulfate

The in-situ methylation of TCA with dimethylsulfate [$(H_3CO)_2SO_2$] requires an ion-pairing agent as modifier. Hereby, the ion-pairing agent improves the yields of TCA-OMe. To a water sample of TCA Na_2SO_4, tetrabutylammonium hydrogensulfate (TBA-HSO_4), and dimethylsulfate are added. In a few minutes TCA-OMe is formed and can be determined by GC/MS. Using an ion-pairing agent it is possible to obtain higher reaction yields. The limit of detection is 1 μg/L (Neitzel, et. al, 1998).

3.6.6 Pentafluorobenzyl bromide (PFBBr)

As PFBBr is a strong lachrymator it should be handled with appropriate precautions. To form pentafluorobenzyl esters of haloacetic acids, samples are re-fluxed with PFBBr and K_2CO_3 in acetone. After cooling and evaporating acetone, the sample is dissolved in hexane and analyzed by gas chromatography/electron-impact ionization/mass spectrometry (GC/EI/MS). In case of TCA (different from other haloacetic acids), 1,1,1-trichloro-2-pentafluorophenyl-ethane is formed instead of the pentafluorobenzyl ester, and then partly degraded to 1,1-dichloro-2-pentafluoro-phenyl-ethene. ECD response for pentafluorobenzyl derivates of organic acids is higher than FID response (Kawahara, 1971; Sinkkonen et al., 1995).

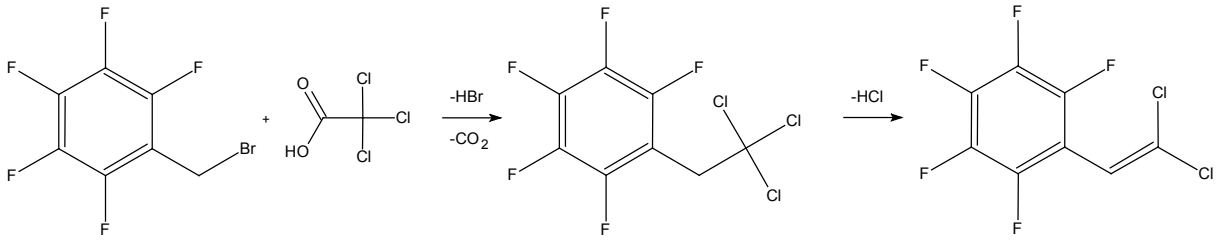

Fig. 9: Derivatization of trichloroacetate with PFBBr (Sinkonnen et al., 2000).

4. ^{14}C isotope (radiocarbon)

4.1 Origin and distribution of radiocarbon

The radioactive ^{14}C nuclide is produced in the atmosphere at a constant rate in a range from ~1 atom/cm^2×s at the equator to 4 atoms/cm^2×s at the poles (Tuniz et al., 1998). Over 99 % radiocarbon is formed in the reaction of atmospheric nitrogen with neutrons originating from the collision of cosmic radiation with air molecules (4.1.). The rest of ^{14}C isotopes in the atmosphere stems mainly from crumbling of the atmospheric oxygen (4.2.), as well as exothermic reaction with neutrons and alpha particles (4.3. and 4.4.) (Lal, 1992, Zito et al., 1980).

$$^{14}N + {}^{1}_{0}n \rightarrow {}^{14}C + {}^{1}H \quad (4.1.)$$

$$^{17}O + {}^{1}_{0}n \rightarrow {}^{14}C + {}^{4}He \quad (4.2.)$$

$$^{13}C + {}^{1}_{0}n \rightarrow {}^{14}C + \gamma \quad (4.3.)$$

$$^{11}B + {}^{4}He \rightarrow {}^{14}C + {}^{1}H \quad (4.4.)$$

^{14}C atoms are oxidized in the atmosphere first to ^{14}CO, then to $^{14}CO_2$ (4.5. and 4.6.) (Conny, 1998) which is distributed in the biosphere due to the photosynthesis process and ocean absorption (Renfrew, 1990).

$$^{14}C + O_2 \rightarrow {}^{14}CO + O \quad (4.5.)$$

$$^{14}CO + OH^{\cdot} \rightarrow {}^{14}CO_2 + H^{\cdot} \quad (4.6.)$$

In this way, radiocarbon becomes part of the carbon dioxide cycle. Atmosphere-biosphere exchange and anaerobic decomposition of organic material introduce radiocarbon into the atmospheric methane cycle.

The equilibrium radiocarbon concentration in the atmosphere strongly increased after thermonuclear bomb tests in the 1960s. Large quantities of bomb ^{14}C are still stored in the atmosphere, biosphere, and hydrosphere. Subsequently, it was released into the atmosphere through aerobic and anaerobic (carbon dioxide and methane, respectively) decomposition of organic material as well as biomass bur-

ning. On the other hand, fossil fuel sources are “negative sources” in the ^{14}C budget, so the disturbed balance stabilizes during time as shown in figure 10.

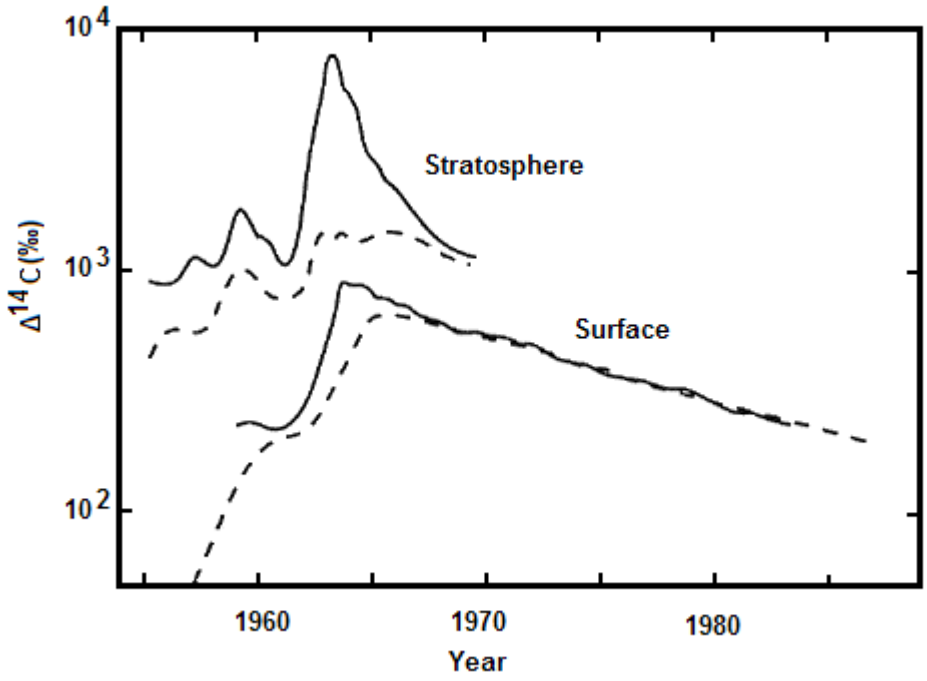

Fig. 10: Long-term atmospheric ^{14}C observations in the northern (solid lines) and southern (dashed lines) hemispheres (Manning et al., 1990).

Radiocarbon decays spontaneously by releasing beta particles, thus forming a nitrogen atom (4.7.).

$$^{14}C \rightarrow {}^{14}N + \beta^{-} \qquad (4.7.)$$

The half-life of the ^{14}C isotope is 5730 years (Conny, 1998; Renfrew, 1990), and its decay rate ~2 atoms/cm^2 x s (Lingenfelter, 1963). Radiocarbon which is trapped in the earth’s crust in organic substances present in natural gas, crude oil and coal, decays during million of years. That is why these deposits contain no ^{14}C.

4.2 Environmental science application of the radiocarbon method

The radiocarbon method was developed by Willard Frank Libby, who was awarded the Nobel Prize in 1960 (Taylor et al., 1992). The base of this method is that modern materials derived from atmospheric carbon dioxide assimilation contain the equilibrium radiocarbon concentration ($^{14}C/^{12}C = 1.2 \times 10^{-12}$) while fossil materials contain no radiocarbon due to its relatively short lifetime. Nowadays, the radiocarbon method is applied for a wide spectrum of samples like air, bone, hair, ice, pollen, pottery, seawater, soil, teeth or wood, in order to determine their age or origin (Tuniz et al., 1998); its most important applications are listed below (Kutschera, 1997; Kretschmer, 2000):

- chemistry, transport and origin of trace gases (carbon monoxide, carbon dioxide, methane);
- transport and origin of biomass burning aerosols;
- cosmic ray production of radionuclides;
- radiocarbon dating;
- in-vivo tracer studies in animals and humans;
- galactic cosmic-ray record in meteorites;
- source identification of environmental chemicals.

4.3 Equipment used for radiocarbon measurement

There are several methods used for radiocarbon measurement either based on decay counting or on atom counting. However, atom counting requires smaller sample sizes, ensures shorter analysis time, and higher precision than decay counting (Gove, 1992).

4.3.1 Gas proportional counting

Gas proportional counting is an indirect method where the sample activity is measured, not the number of ^{14}C atoms. The sample is converted into carbon dioxide or methane which is then used to fill the gas counter. However, carbon dioxide is the primary gas produced in all methods. It is easily obtained in high purity and used

more often than methane (Kromer et al., 1992). In the counter the decay of radiocarbon causes an electrical discharge of the gas which is electronically detected. The decay rate depends on the number of ^{14}C atoms present in the sample.

This method is easy to handle, relatively cheap, and the long-term drift of the equipment is very small such ensuring high accuracy of measurement. Samples combusted to carbon dioxide can be used directly which eliminates additional contamination sources and minimizes isotope fractionation. However, the amount of carbon required is between 10 mg and a few grams. Furthermore, the activity of one mole of modern carbon amounts to three decays per second which is very low. This leads to very long measurement periods, sometimes up to a few weeks, especially for old samples containing only small amounts of ^{14}C. Additionally, samples older than 40,000 before present (BP) cannot be measured due to their small counting rate which cannot be separated from the cosmic background radiation (Uhl, 2004).

4.3.2 Liquid scintillation (LS) counting

LS counting is also an indirect method used for radiocarbon analysis to measure the sample activity. The sample is combusted to carbon dioxide and mixed with metallic lithium to form lithium carbide which is hydrolyzed to acetylene and subsequently converted into benzene by catalytic trimerization. Benzene is mixed with a scintillation liquid diluted in toluene and placed in a cuvette. Due to the beta decay of radiocarbon, the solution is ionized and emits photons as a pulse of light which is detected by photomultiplier tubes. The signal is proportional to the energy of ionized particles.

This method is relatively cheap and stable over a long period of time. It is possible to measure different samples repeatedly and independently by simply changing the cuvette. On the other hand, the sample preparation process is complicated and can be a source of contaminants. As in the case of gas proportional counting, large sample amounts are required and the measurements take a long time. This method is also not suitable for samples older than 40,000 BP due to their very small activity (Uhl, 2004).

4.3.3 Accelerator mass spectrometry (AMS)

AMS is the most sensitive direct technique for radiocarbon analysis. Atoms extracted from a sample are ionized, accelerated to high energies, separated due to their momentum, charge and energy, and individually counted. There is no need to measure the decay rate as in the case of indirect methods.

The goal of the sample preparation procedure for AMS measurement is to get a sufficient amount of sample in the purest form possible. All contamination sources have to be minimized in order to obtain reliable results. Also fractionation of the carbon isotopes, taking place in the natural environment of the sample or in the laboratory, has to be taken into account. Overall the sample preparation procedure comprises the following steps (Tuniz et al., 1998):

- removal of non-essential material, preliminary cleaning and mineral separation;
- dissolution and addition of carrier if necessary;
- separation of the analyte;
- conversion of the sample by combusting it with copper oxide and silver wool gaining carbon in a form suitable for AMS (graphite or CO_2).

4.3.3.1 Classical approach to AMS-target preparation

Using the classical ion source of an AMS instrument, CO_2 derived from samples has to be further reduced to graphite by heating it with hydrogen and iron powder, followed by pressing it into a sputter target under pressure (Kretschmer et al., 1997):

$$2\ Cl_3CCOOCH_3 + 5\ O_2 + CuO \xrightarrow{600\ °C} 6\ CO_2 + 3\ Cl_2 + 3\ H_2O + Cu \qquad (4.8.)$$

$$CO_2 + 2\ H_2 + Fe \xrightarrow{625\ °C} 2\ H_2O + Fe/C\ (target) \qquad (4.9.)$$

4.3.3.2 Modified approach to AMS-target preparation

There is also the possibility to modify the AMS ion source to measure gas samples (CO_2) directly without converting them into graphite targets first. This modifi-

cation allows to lead CO_2 obtained from sample combustion directly into the ion source onto a titanium pellet which constitutes a reactive surface, where it is adsorbed and reduced. Subsequently, the ionization by means of a Cs-sputter source is the same as in a graphite ion source.

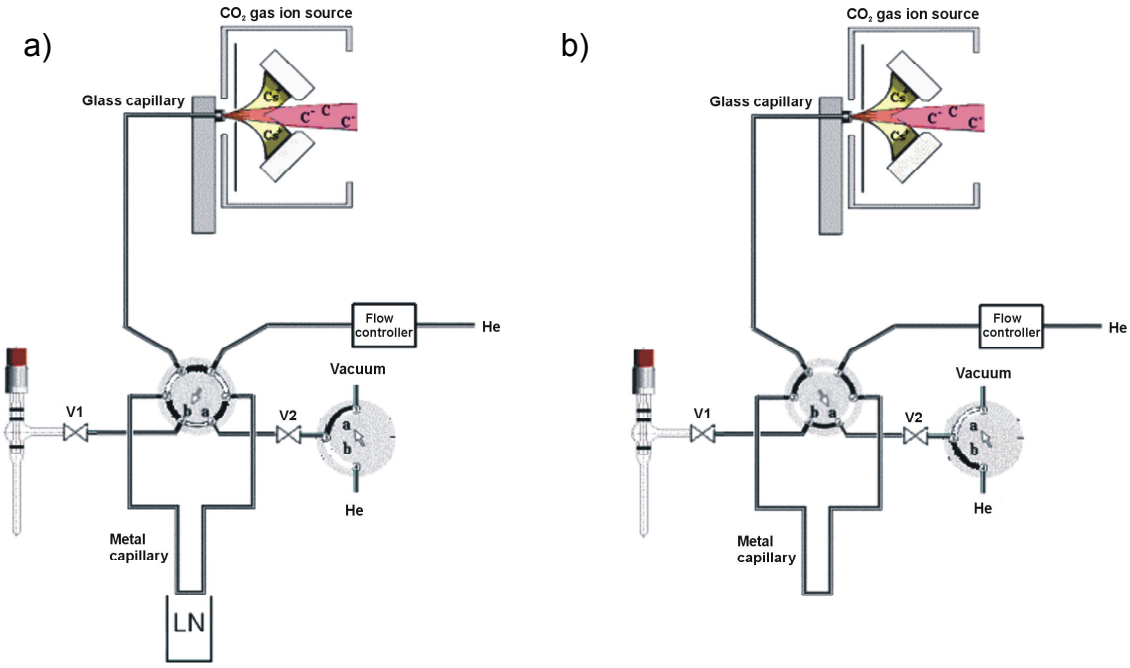

Fig. 11: Schematic diagram of an AMS gas ion source: a) pumping and cryogenic transfer of CO_2 to reservoir; b) measurement; V1, V2: valves, LN: liquid nitrogen (Kretschmer et al. 2004).

The gas-tight cold-finger filled with CO_2 is connected to a gas handling system and CO_2 is lead across into a metal capillary (figure 11a) from where it is transported with a low helium flow into the ion source (figure 11b). This technical modification increases the ionization yield and reduces the amount of carbon required for analysis from originally 30 μg to 1 - 5 μg per sample.

4.3.3.3 AMS measurement

In figure 12 schemes of conventional MS and AMS are depicted for comparison (Tuniz et al., 1998).

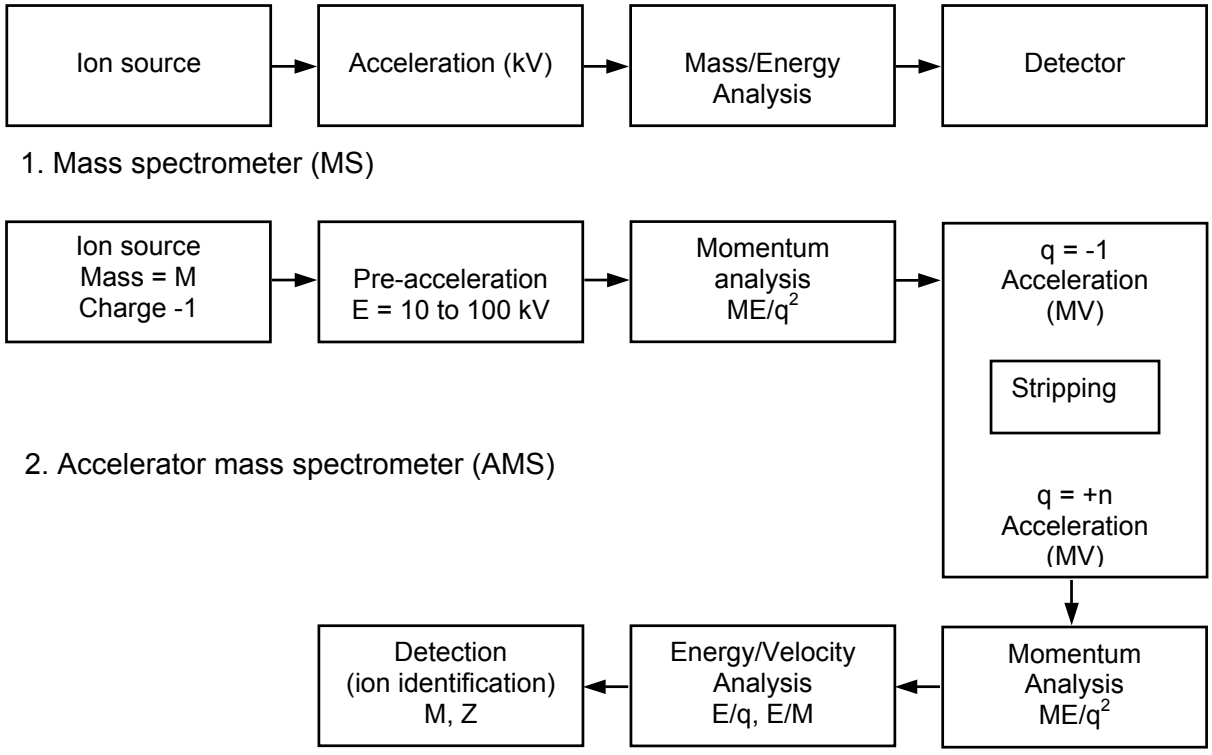

Fig. 12: Scheme of MS and AMS (M-mass, E-energy, q-charge, kV-kilovolt, MV-megavolt) (Tuniz et al., 1998).

An AMS system consists of:

- injector with negative ion source and electrostatic analyzer for selecting ions with defined energy;
- mass spectrometer for selecting ions with defined ME/q^2 value;
- tandem accelerator for accelerating ions to high energies;
- stripper located in the high-voltage terminal of the accelerator for converting negative ions into positive ions;
- magnetic and electrostatic sectors after the accelerator for further rejecting background ions;
- final detector for identifying the ions with defined mass and atomic number;
- faraday cups for measurement of currents of stable isotopes.

The main difference between AMS and conventional MS is that in MS ions are accelerated to much lower energies (1 keV) than in AMS (a few MeV). Consequently, applying AMS high energy isobars and molecular ions (e.g. ^{14}N, ^{13}CH, $^{12}CH_2$) can be identified and isolated due to nuclear mass and charge identification. Multiple elec-

tron stripping and applying a second stage of acceleration followed by identification of individual ions ensures the complete rejection of molecular background. Such isotopic ratios can be determined in the range of 10^{-12} to 10^{-15}. As this method is independent of the counting rate even samples with very small activities up to 50,000 BP can be measured. AMS measurements take between a few minutes and hours. Moreover, the sensitivity can be obtained for sample amounts smaller than 1 mg. The AMS detection limit depends on the ion beams producing efficiency, background control in the system, and the sample contamination level (Tuniz et al., 1998).

Nevertheless, AMS poses some challenges: the equipment is very expensive; sample preparation procedures are mostly very complicated and time-consuming as ultra-clean levels are required due to the very low AMS background capabilities (Kutschera, 1997). Moreover, the experimental setup is not stable over a long period of time and requires frequent calibration (Uhl, 2004).

4.3.3.4 Results of AMS measurement

^{14}C contents are given in the unit percent modern carbon (PMC). In 1957, oxalic acid was produced which is used as calibration standard. It was established that 95 % of the ^{14}C concentration of this substance correlates to 100 PMC. The PMC value of a real sample is obtained by comparing its $^{14}C/^{12}C$ ratio with a calibration standard which PMC content is known.

100 PMC ≡ 95 % NBS-Ox I (4.10)

or 100 PMC ≡ 74.6 % NBS-Ox II (4.11)

NBS-Ox I, NBS-Ox II - oxalic acids as calibration standards
NBS - National Bureau of Standards

5. Kovats retention index

The retention index is a useful tool for the comparison of retention data obtained under different conditions during gas chromatographic analysis. It assures independence of parameters like column length and carrier gas flow rate (Castello, 1999). This is achieved by relating the retention time of the investigated compound to those of n-alkanes. The latter are suitable for this purpose as within a homologous series of n-alkanes a linear relationship exists between the logarithm of the adjusted retention time and the number of carbon atoms in the compound. Practically, the analyte of interest is co-injected with at least two n-alkanes with known retention indices which elute before and after the compound of interest. Based on the data obtained from the resulting gas chromatogram the retention index of the compound of interest is calculated according to the following equation (Castello, 1999; Mermet et al., 1998):

$$I_s^{st.ph.}(T) = 100\left(\frac{\log t'_{Rs} - \log t'_{Rz}}{\log t'_{R(z+1)} - \log t'_{Rz}}\right) + 100z \qquad (5.1.)$$

I: isothermal retention index at temperature T

s: compound of interest

st. ph. : stationary phase

z: carbon number for the n-alkane with lower content of carbon atoms

t'_{Rs}: retention value relevant for the compound of interest [s]

t'_{Rz}: retention value relevant for the n-alkane with z carbon atoms [s]

$t'_{R(z+1)}$: retention value relevant for the n-alkane with carbon atoms z+1 [s]

Thereby, the retention values are obtained by subtracting the dead time of the analytical column from the retention time of a given compound.

As the retention values differ with the temperature of the column, it is tricky to predict the retention index of a given compound at another temperature as the temperature dependence is hyperbolic, and the error in values obtained by linear extrapolation might be considerably. Therefore, equation 5.1. cannot be used in temperature-programmed gas chromatography (TPGC) analysis. The latter problem

was solved using an arithmetic relationship similar to the logarithmic one of Kovats (Castello, 1999):

$$I_{TPGC,s}^{st.ph.} = 100n\left(\frac{t_{Rs} - t_{Rz}}{t_{R(z+n)} - t_{Rz}}\right) + 100z \qquad (5.2.)$$

I: retention index

n: difference in carbon number of the two n-alkanes taken as references

s: compound of interest

st. ph. : stationary phase

z: carbon number for the n-alkane with lower content of carbon atoms

t_{Rs}: retention time of the compound of interest [s]

t_{Rz}: retention time of the n-alkane with z carbon atoms [s]

$t_{R(z+n)}$: retention time of the n-alkane with carbon atoms z+n [s]

In TPGC equation 5.2. is used to calculate retention indices. It is strictly valid when a linear correlation exists between the retention times of n-alkanes and the number of carbon atoms. As opposed to the logarithmic relation the arithmetic one uses gross retention times instead of retention values. One advantage of this approach is that dead time measurements are not necessary. On the other hand, large errors may occur when the analyte eluting first is delayed as a consequence of carrier gas hold-up or when multilinear temperature programming is set (Castello, 1999).

6. Experimental work

6.1 Chemicals and equipment

6.1.1 Chemicals

Chemicals used are listed here:

- 2,2-dichloropropionic acid, 95 %, Fluka Chemie, Buchs, Switzerland,
- 2,3-dichlorpropionic acid, 95 %, Fluka Chemie, Buchs, Switzerland,
- n-hexane, ≥ 99 %, Merck, Darmstadt, Germany,
- n-paraffins mixture C9 - C12, Alltech, Mainz, Germany,
- N-nitroso-N-methyl-4-toluenesulfonamide, 99 %, Fluka Chemie, Buchs, Switzerland,
- acetic acid, ≥ 99.7 %, Roth, Karlsruhe, Germany,
- acetone, ≥ 99.0 %, Promochem, Wesel, Germany,
- activated charcoal granulated, 1.5 mm purest, Merck, Darmstadt, Germany,
- calcium chloride, ≥ 97 %, Fluka Chemie, Buchs, Switzerland,
- chlorotrimethylsilane, ≥ 99.0 %,Fluka Chemie, Buchs, Switzerland,
- diethyl ether, > 99.5 %, Merck, Darmstadt, Germany,
- dry ice,
- glass fiber filter, GF 8, 120 mm, Schleicher & Schuell, Dassel, Germany,
- glass wool, Roth, Karlsruhe, Germany,
- ice,
- liquid nitrogen, Linde, Höllriegelsreuth, Germany,
- Maxigas 400 (n-butane, isobutane, propene), Rothenberger, Kelkheim, Germany,
- methanol, ≥ 99.0 %, Promochem, Wesel, Germany,
- methyl-*t*-butyl ether, > 98 %, Fluka Chemie, Buchs, Switzerland,
- oxygen, Rothenberger, Kelkheim, Germany,
- 1-pentafluorophenyl-diazoethane, synthesized according to Meese (Meese, 1985),
- potassium hydroxide, ≥ 85 %, Merck, Darmstadt, Germany,
- silica gel, 70 - 230 mesh, Merck, Darmstadt, Germany,
- sodium carbonate anhydrous, > 99.5 %, Fluka Chemie, Buchs, Switzerland,

- sodium chloride, > 99.5 %, Fluka Chemie, Buchs, Switzerland,
- sodium hydroxide, ≥ 99 %, Merck, Darmstadt, Germany,
- sodium pure in kerosene, Fluka Chemie, Buchs, Switzerland,
- sodium sulfate anhydrous, ≥ 99.0 %, Merck, Darmstadt, Germany,
- sulfuric acid, 95 - 97 %, Fluka Chemie, Buchs, Switzerland,
- toluene, ≥ 99.5 %, Merck, Darmstadt, Germany,
- trichloroacetic acid, > 99.5 %, Fluka Chemie, Buchs, Switzerland,
- trichloroacetic acid methyl ester, > 99 %, Aldrich-Chemie, Steinheim, Germany.

6.1.2 Equipment

6.1.2.1 GC-MS

The chromatographic system used for quantification consists of a gas chromatograph (HP 5890 II, Hewlett Packard, Waldbronn, Germany), an autosampler (200 S, Fisons Instruments, Mainz, Germany), and a mass spectrometer (HP 5989 A, MS-Engine).

6.1.2.2 PC-GC

The chromatographic system used for preparative separation (figure 13) consists of a single-oven gas chromatograph with FID detector (HP 5890 II, Hewlett Packard, Waldbronn, Germany), an autosampler (Gerstel, Mülheim, Germany), a large volume injector (Gerstel, Mülheim, Germany), a cold injection system (Gerstel, Mülheim, Germany), and a preparative fraction collector (Gerstel, Mülheim, Germany). The two-stage separation is performed by an off-line procedure whereby both separation steps are carried out consecutively on the same single-oven instrument.

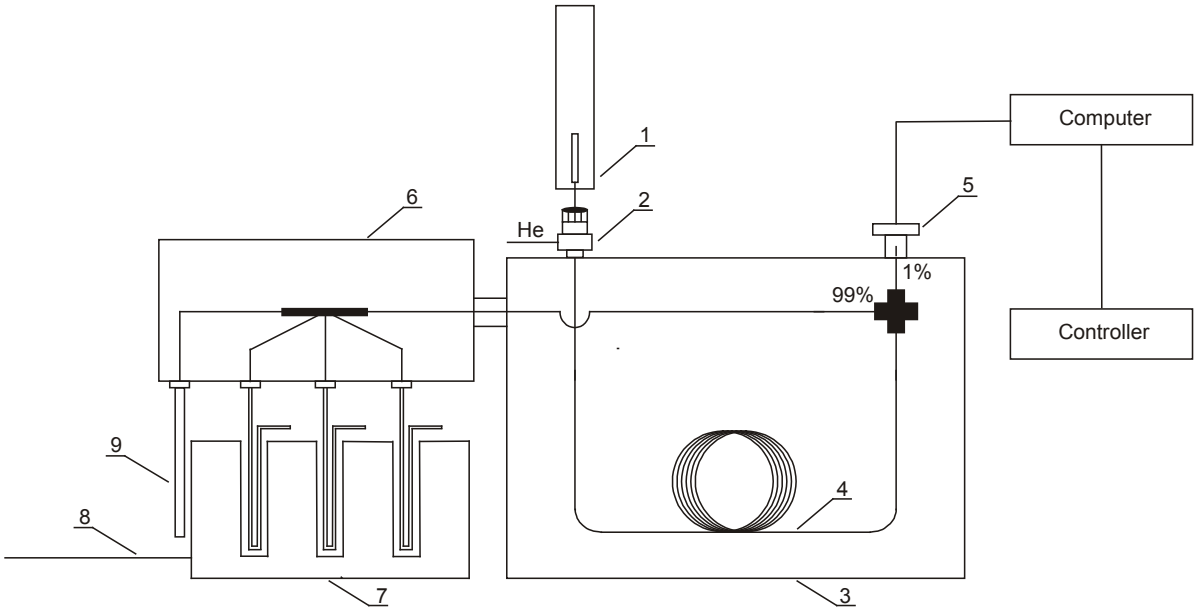

Fig. 13: Gas chromatograph with large volume injector and cold injection system: 1 - autosampler, 2 - cold injection system, 3 - gas chromatograph, 4 - capillary column, 5 - flame ionization detector, 6 - preparative fraction collector, 7 - analyte traps, 8 - cooling medium (liquid nitrogen), 9 - waste trap.

The large volume injector allows injections of up to 1000 μL per sample and the cold injection system is controlled by a temperature regulator which let the solvent evaporate from the injector before the sample is led to the column. In this injection mode, called solvent vent-stop flow, the split is initially open and a sample is introduced into a Tenax-filled glass liner. The column head pressure is dropped to atmospheric pressure and before the inlet is heated, the solvent is vented at a temperature of about 30 °C below its boiling point. After all solvent is vented, the split is closed, the inlet is rapidly heated and the sample retained in the liner is transferred onto the analytical column. After separation on the column, 1 % of the stream of analytes is led to the detector via a fused-silica capillary (71 mm x 0.05 mm i.d.). The remaining 99 % are led to the preparative fraction collector via a fused-silica capillary (870 mm x 0.32 mm i.d.). The interface, connecting gas chromatograph and preparative fraction collector, is heated to 320 °C. In six glass traps, each with a volume of 1 μL, six different analytes can be collected; one trap with a volume of 100 μL is used to collect waste. All traps are cooled with liquid nitrogen. All elements are controlled by an electronic timing unit (Gerstel 505, Gerstel, Mülheim, Germany).

6.1.2.3 AMS

The AMS facility (figure 14) in Erlangen used for radiocarbon determination consists of a sputter ion source (40 MGF-SNICS; Multiple Gas Feed - Source of Negative Ions by Cesium Sputtering, NEC), a tandem accelerator (model EN, HVEC), mass analyzers, and detectors.

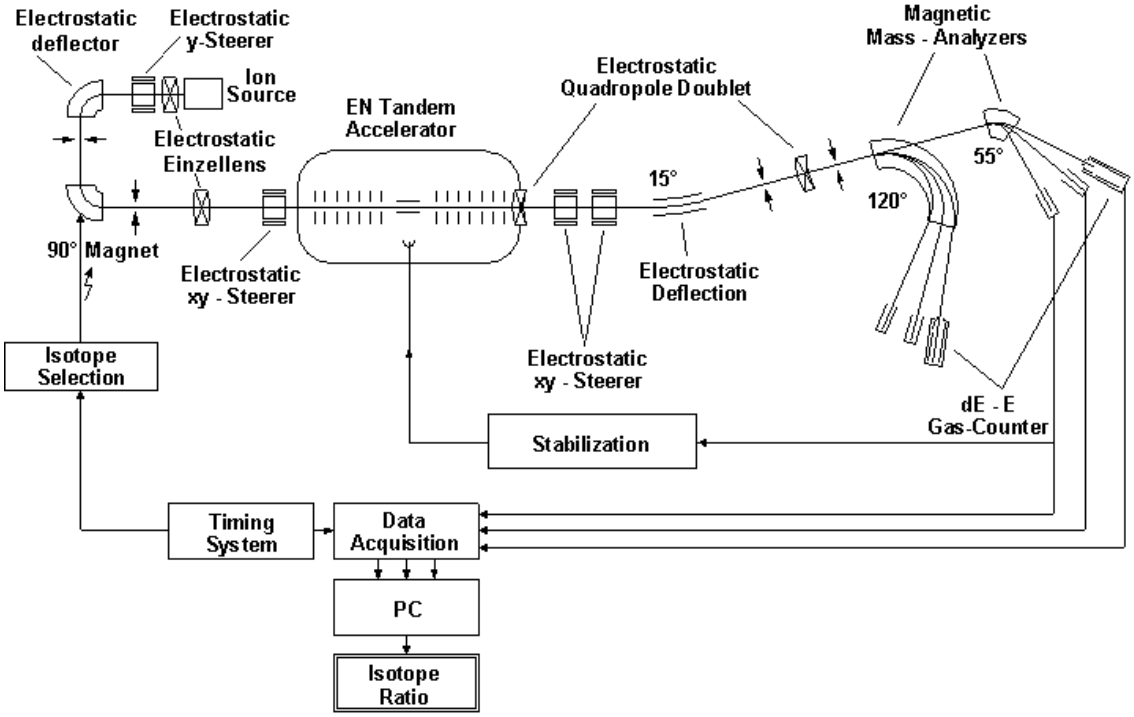

Fig. 14: Schematic diagram of an AMS facility.

Targets are positioned in the ion source of an AMS where negatively charged carbon ions are generated. The latter are separated due to their energy, mass, and electrical charge and accelerated towards the terminal (+5 MV) where they hit a thin carbon foil and change their polarity through stripping of the outer shell electrons. The four-fold positively charged carbon ions are then accelerated through the second stage of the accelerator reaching kinetic energies of the order of 25 MeV. They are directed from the terminal and leave the accelerator. Later on they are separated due to their charge and mass. In the gas ionization detector ^{14}C particles are measured. By measuring the total energy loss E and the so called differential energy loss dE (total energy of an incoming ion and the rate at which it slows down as it passes

through the gas-filled detector) ^{14}C particles can be differentiated from the background. ^{13}C and ^{12}C are measured in Faraday cups as charge current.

Besides carbon ions, disturbing particles such as $^{12}CH_2^-$ or $^{13}CH^-$ are also formed in the ion source. These particles have the same mass as $^{14}C^-$ but are destroyed in the accelerator through stripping.

6.1.2.4 Other equipment

Other equipment used is listed here:

- 250 mL PP-bottles, Nalgene, Nalge Company, New York, USA,
- 4 L PP-bottles, Nalgene, Nalge Company, New York, USA,
- 50 mL PP-centrifugal cones, Nalgene, Nalge Company, New York, USA,
- ball mill, MM 2000, Retsch, Haan, Germany,
- brazing and welding set, Rothenberger Roxy 400L, Kelkheim, Germany,
- centrifuge, Avanti J-25, Beckman Instruments, California, USA,
- horizontal shaker, Edmund Büchler, Tübingen, Germany,
- lyophilizer, Edwards, Crawley, Sussex, England,
- muffle oven, Oven Carbolite, Sheffield, England,
- overhead shaker, own construction,
- rotary evaporator, Rotavapor, Büchi, Flawil, Switzerland,
- sieve, mesh size 2 mm, Retsch, Haan, Germany,
- ultrasonic bath, Sonorex RK 510 H, Bandelin, Germany,
- Vortex-shaker, Heidolph, Schwabach, Germany.

6.2 Sampling site selection

Environmental concentrations of TCA vary strongly even within one compartment. For example, during European forest soil monitoring project of the Netherlands Organization for Applied Scientific Research (TNO), different concentrations of TCA were detected at different sampling places but no unequivocal dependence of TCA concentration on the depth of sampling was found (see figure 15). The concentrations also vary strongly in Europe, i.e. between 0.05 μg/kg in Göteborg, Oslo, Rotterdam and Glasgow and 12 μg/kg in Freudenstadt. As the amount of soil

required for radiocarbon analysis depends, inter alia, on the actual TCA concentration several monitoring projects carried out all over Europe were consulted to select a suitable sampling site.

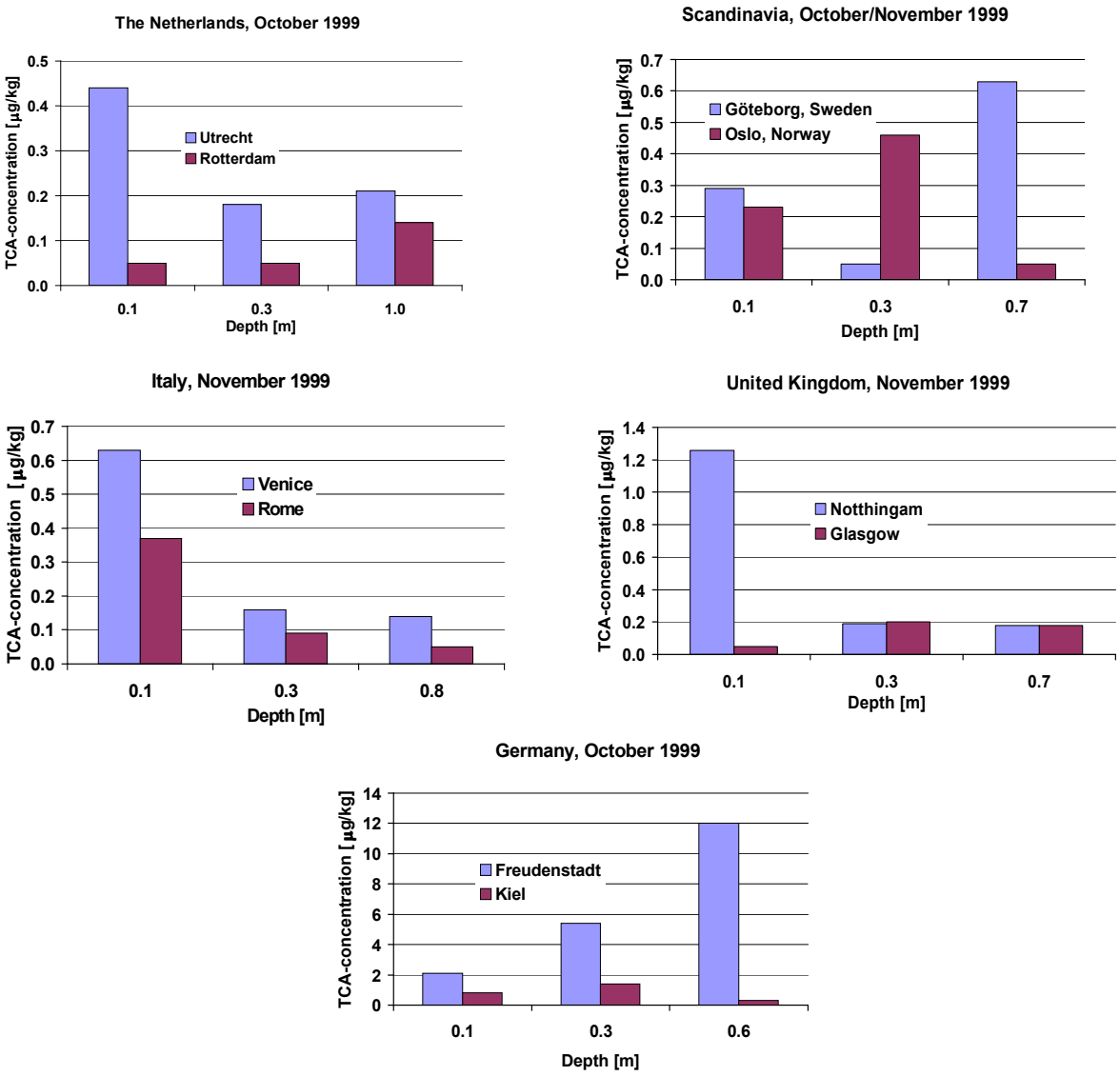

Fig. 15: TCA-concentrations in forest soil in different sampling places in Europe: the Netherlands, Scandinavia, Italy, United Kingdom and Germany in 1999 (data from Peters, 2000).

Finally, a study on TCA levels at the sites of the Bavarian spruce site observational network (LfU) where TCA concentrations in needles were determined from 1992 to 1995 was used to find a sampling place exhibiting high TCA levels in soil (data are shown in figure 16).

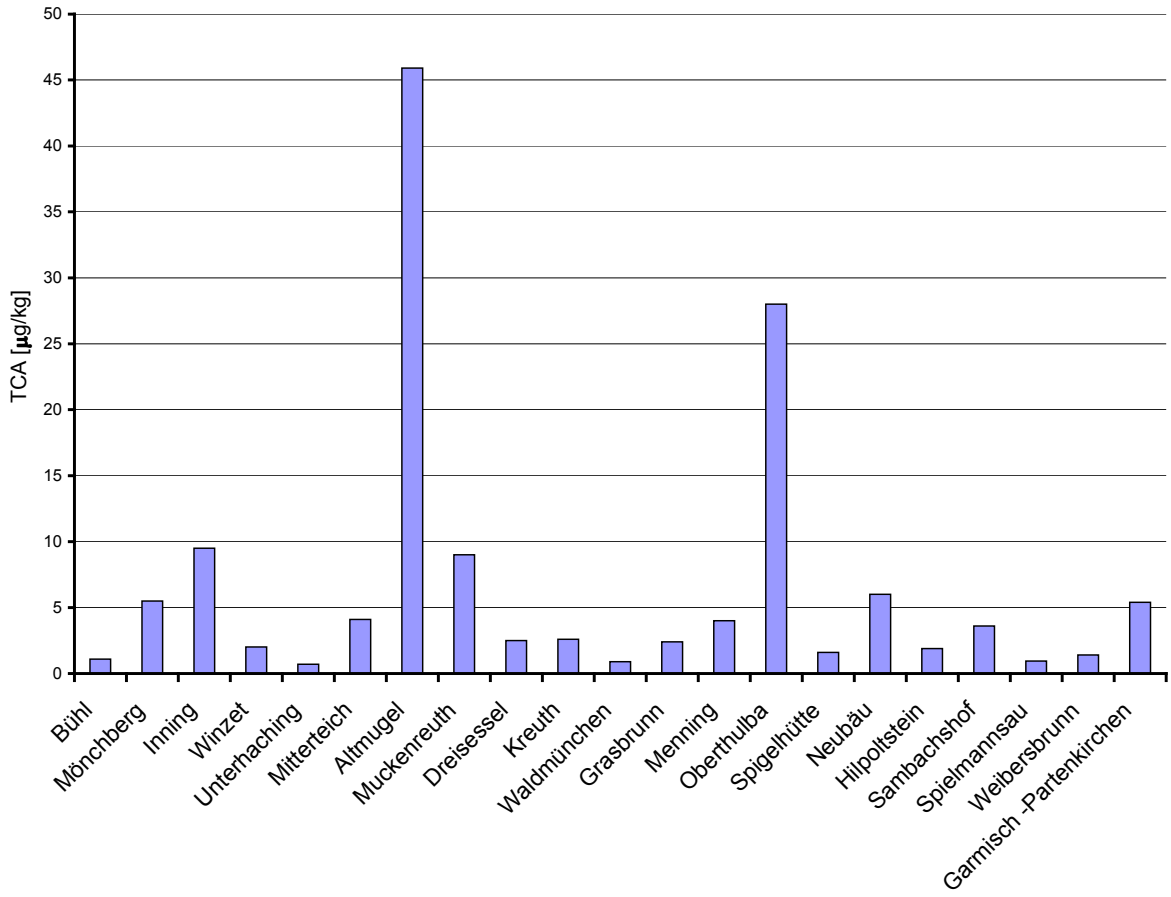

Fig. 16: TCA concentrations in spruce needles in LfU-observational network from 1992 to 1995 (Frank et al., 1997).

The highest TCA concentrations in spruce needles are found in Oberthulba (north-western Bavaria, coordinates: 35°72', 55°66', 320 m above sea level) and Altmugel (eastern Bavaria, coordinates: 45°32', 55°33', 700 m above sea level): 28 µg/kg and 46 µg/kg, respectively. Consequently, as it is known that the main TCA transport pathway to spruce needles leads through the root system (Matucha et al., 2001) and that soils under coniferous trees may contain elevated TCA levels (Schöler et al., 2003b), it seems most likely that TCA concentrations in soil at these sampling sites are also high. On 5 June 2002 (Oberthulba) and on 11 June 2002 (Altmugel), 0.5 kg samples both of the organic (5 - 10 cm in Oberthulba and 10 - 20 cm in Altmugel) and the mineral (10 - 20 cm in Oberthulba and 20 - 30 cm in Altmugel) soil horizons were taken and transported to the laboratory in polypropylene (PP)-bags. There, the TCA concentrations were determined and such a sampling site with a high TCA burden was identified.

The highest TCA concentrations are found in the mineral layer in Oberthulba. At a TCA concentration of 3 μg/kg dw, 70 kg of soil has to be taken to isolate ca. 30 μg carbon which were necessary for AMS-measurement in the beginning of the study.

$$m_B = \frac{m_C \cdot M_{TCA}}{2 \cdot c_{TCA} \cdot M_C} \qquad (6.1.)$$

m_B, m_C: soil mass [kg] and carbon mass required for AMS [μg], respectively

c_{TCA}: TCA concentration in soil [μg/kg], (2 carbon atoms per TCA molecule)

M_{TCA}, M_C: TCA molar mass and carbon molar mass, respectively [g/mol]

To account for possible incomplete extraction and taking the water content of soil into consideration, over 100 kg of wet soil were collected at Oberthulba on 25 July 2002. In 6 squares of 1 m x 1 m each (spread across a circle of ~30 m) the mineral layer was collected in PP-bags (ca. 4 kg per bag: 300 mm x 500 mm), transported to the laboratory at the same day, and stored at -30 °C until further work up.

The sampling site in Oberthulba is situated at the south-west edge of a spruce forest. The rainfall pattern at the meteorological station Maria-Bildhausen (triangle, figure 17) 30 km north-east of the sampling site during the month prior to sampling is depicted in figure 18.

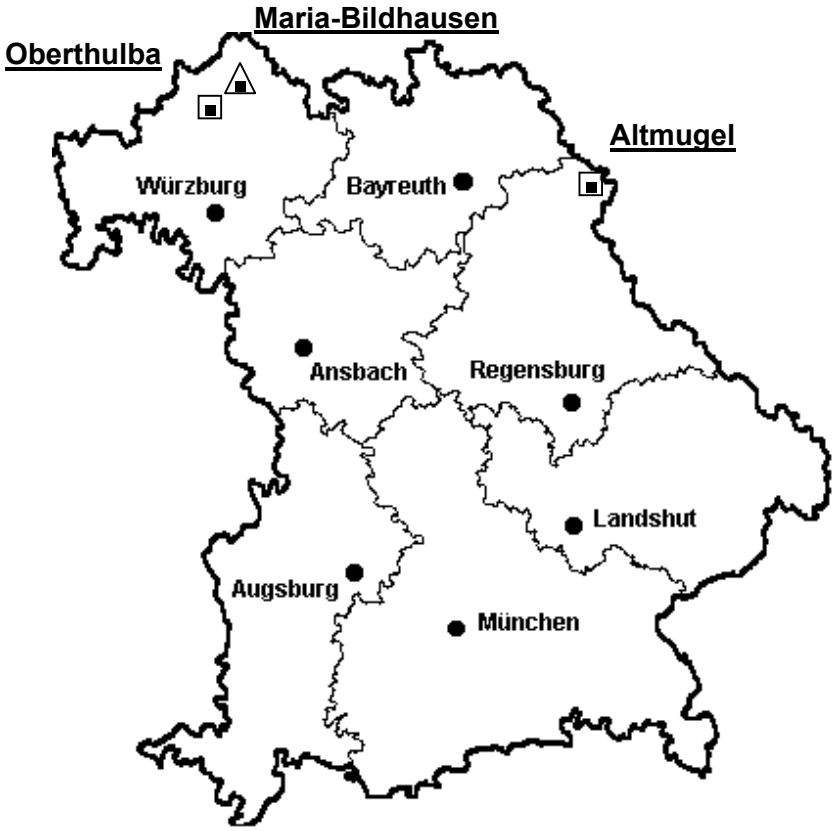

Fig. 17: Sampling sites ▣ and meteorological station Maria-Bildhausen ◬ .

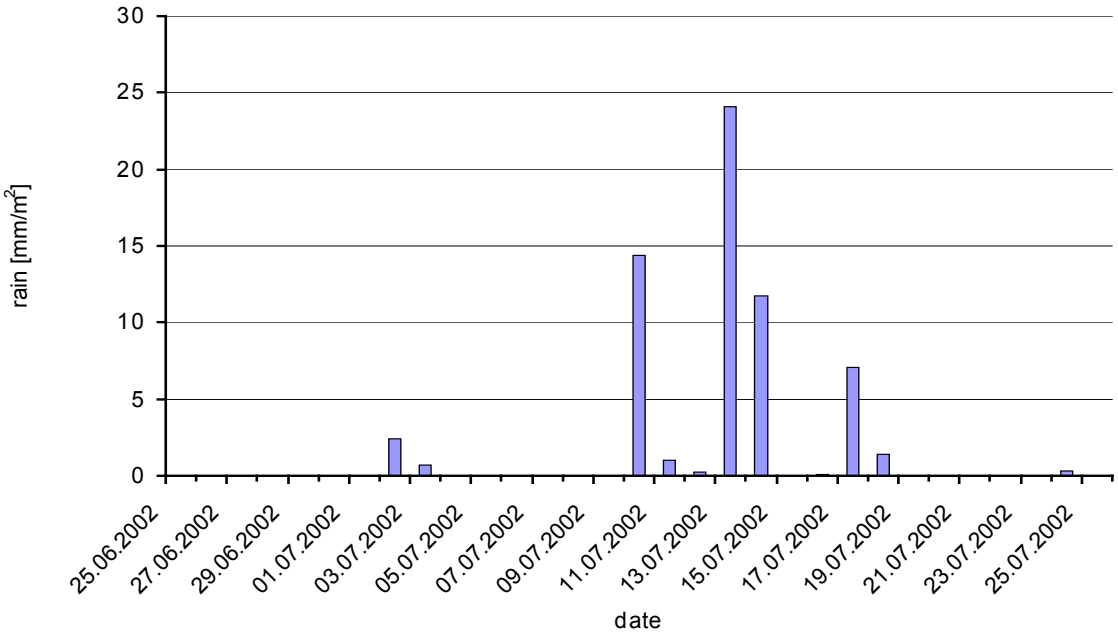

Fig. 18: Rainfall during the month prior to sampling (Maria-Bildhausen, www.stmlf-design2.bayern.de).

6.3 Cleaning of glassware and PP-equipment, removal of contaminants

PP-bottles (4 L) used for extraction and PP-centrifuge cones (250 mL) are washed first with deionized water, then with NaOH (1 mol/L), deionized water, H_2SO_4 (1 mol/L), deionized water, and finally acetone.

All glass vials used for quantification as well as for preparative analysis are silanized. First they are washed with deionized water and dried, then kept in a solution of 5 % trimethylchlorosilane in toluene for 24 hours. Afterwards they are washed twice with methanol and twice with MTBE (for quantification) or diethyl ether (Et_2O) (for preparative analysis), respectively. Dry vials are stored in a clean bench.

All operations are performed in a closed hood filled with a circulating nitrogen atmosphere purified by passing through KOH pellets and a layer of activated charcoal.

6.4 Quantitative determination of TCA in soil

The collected soil is sieved (mesh size 2 mm) and water content (50 g soil are dried at 105 °C until constant mass is reached) and pH values (in aqueous 0.01 mol/L $CaCl_2$ solution) are measured.

The concentrations of TCA are determined after derivatization to the corresponding pentafluorophenyl-ethyl ester by GC/NCI/MS. Sample preparation is shown in figure 19.

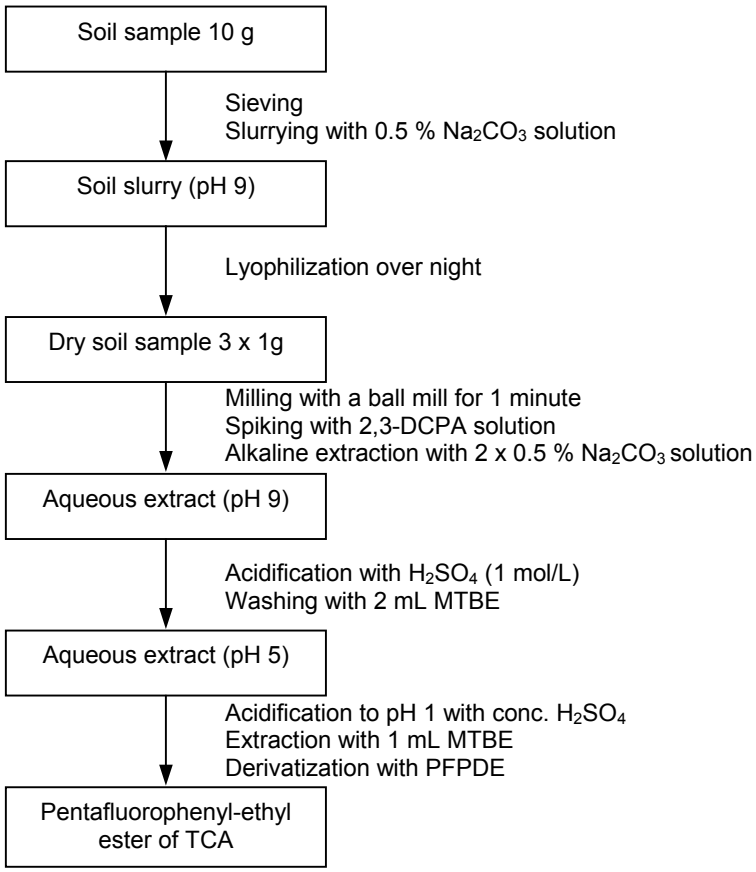

Fig. 19: Sample preparation for GC/NCI/MS analysis.

For quantitative analysis, soil aliquots of 10 g are taken. The soil is slurried in 250 mL PP-bottles with aqueous 0.5 % Na_2CO_3 solution which is added (10 - 20 mL) until pH 9 is reached. Samples are frozen in liquid nitrogen and lyophilized overnight. Dry samples are milled in a ball mill for 1 minute; three aliquots of 1 g soil powder are weighed into 50 mL PP-centrifuge cones and 6.1 ng 2,3-dichloropropionic acid (2,3-DCPA; in 50 μL aqueous solution) are added as internal standard. The aliquots are slurried with 5 mL 0.5 % Na_2CO_3 solution, shaken for 1 minute with a Vortex-shaker, placed into an ultrasonic bath for 10 minutes, and subsequently mixed in a horizontal shaker at 350 rev min^{-1} for 10 minutes. Samples are centrifuged at 15 °C with a speed of 14,000 rotations per minute for 12 minutes. Aqueous supernatants are transferred to new PP-centrifuge cones and the extraction is repeated with another portion of 5 mL 0.5 % Na_2CO_3 solution. Supernatants are combined, acidified with H_2SO_4 (1 mol/L) to pH 5, shaken with 2 mL MTBE in a Vortex-shaker for 1 minute, and centrifuged for 12 minutes. The ether layers are discarded and the extraction step is repeated until the ether layer is clean and colorless; mostly two extraction

steps are sufficient. To the water phase 1 g NaCl, 100 μL concentrated H_2SO_4 to reach pH 1, and 1 mL MTBE are added. After shaking for 1 minute with a Vortex-shaker, aqueous and organic phases are separated by centrifugation for 12 minutes. Ether phases are transferred into 1 mL vials and 8 μL of a solution of 40 μL PFPDE in 0.5 mL MTBE are added to derivatize TCA to its corresponding PFPDE ester (12 hours, room temperature 23 °C). Anhydrous Na_2SO_4, about 80 mg, is added to each sample for drying. After shaking the vials TCA is determined by GC/NCI/MS applying the conditions as given in table 3.

Simultaneously, standards for a calibration curve are prepared. Three times the aliquots of 10, 50, 100, 150, 200, 250, 300, 350, and 400 μL of TCA-Na water solution (13.6 ng/mL) are added to 10 mL of 0.5 % Na_2CO_3 solution and also 6.1 ng 2,3-DCPA (in 50 μL aqueous solution) as internal standard. The solutions are acidified with H_2SO_4 (1 mol/L) to pH 5 and after adding 1 g NaCl the further work-up follows as described for soil samples.

Gas chromatography	
Pre-column	0.5 m x 0.53 mm deactivated fused silica capillary (Varian-Chrompack)
Column, stationary phase	95 %-methyl -5 %-phenyl-polysiloxane 0.25 μm film thickness (CP-Sil 8 CB-MS, Varian-Chrompack)
Column, dimensions	15 m x 0.25 mm
Carrier gas	helium 4.6; head pressure 70 kPa
Injection / sample aliquot	on column; 60 °C; 1.0 μL
Temperature program	60 °C, 1.5 min isotherm; 25 °C min^{-1} to 240 °C; 1.3 min isotherm
Interface temperature	240 °C
Mass spectrometry	
Ionization mode	negative chemical ionization (NCI)
Reactant gas	methane 5.5
Electron energy, anode current	230 eV, 70 μA
SEV-voltage	2000 - 2400 V
Ion source temperature	200 °C
Detection mode Selected-ion monitoring (SIM)	m/z 161 (TCA) m/z 141 (2,3-DCPA)

Tab. 3: Gas chromatographic and mass spectrometric conditions for quantitative determination of TCA in soil.

6.5 Optimization of soil extraction process

The extraction conditions are optimized for larger soil amounts. Different extraction agents, extraction times, and the number extraction steps are compared.

6.5.1 Selection of extraction agent

Two extraction agents are compared: deionized water and aqueous 0.5 % Na_2CO_3. Twice, 1 kg soil is sieved (fraction < 2 mm), placed into 4 L PP-bottles, and

610 ng 2,3-DCPA (in 5 mL aqueous solution) are added as internal standard. The aliquots are shaken in an overhead shaker with 1.5 L deionized water or aqueous 0.5 % Na_2CO_3 solution, respectively, for 3 hours. After sedimentation the supernatants are removed, another 1.5 L deionized water or aqueous 0.5 % Na_2CO_3 solution are added, respectively, and the mixtures are shaken again for 3 hours. The supernatants are removed after sedimentation, another liter deionized water or aqueous 0.5 % Na_2CO_3 solution is added, and the mixtures are shaken for yet another 3 hours. After sedimentation the aqueous phases are combined, filtered through a glass filter, transferred into several 250 mL PP-bottles, centrifuged twice at 15 °C with a speed of 10,000 rotations per minute for 6 minutes, and concentrated to an end volume of 1 L under reduced pressure (40 °C, 90 mbar). Three aliquots of 10 mL of each supernatant are acidified with H_2SO_4 (1 mol/L) to pH 5. The following extraction with MTBE, the derivatization with PFPDE, and the determination of TCA concentration is conducted as described in section 6.4.

6.5.2 Optimization of extraction time

Extraction times of 1, 2, and 3 hours are tested. For this purpose, three aliquots of 1 kg soil each are sieved (fraction < 2 mm), placed into 4 L PP-bottles, and in each case 610 ng 2,3-DCPA (in 5 mL aqueous solution) are added as internal standard. The aliquots are shaken in an overhead shaker with 1.5 L aqueous 0.5 % Na_2CO_3 solution for 1, 2, or 3 hours, respectively. After sedimentation the supernatants are removed, 1.5 L aqueous 0.5 % Na_2CO_3 solution are added, and the mixtures are shaken again for 1, 2, or 3 hours, respectively. Once more the supernatants are removed after sedimentation, one liter aqueous 0.5 % Na_2CO_3 solution is added, and the mixtures are shaken again for 1, 2, or 3 hours, respectively. Then the aqueous phases are combined after sedimentation, filtered through a glass filter, transferred into several 250 mL PP-bottles, centrifuged twice at 15 °C with a speed of 10,000 rotations per minute for 6 minutes, and concentrated to an end volume of 1 L under reduced pressure (40 °C, 90 mbar). Three aliquots of 10 mL of each supernatant are acidified with H_2SO_4 (1 mol/L) to pH 5. The following extraction steps and the determination of TCA concentration is accomplished as described in section 6.4.

6.5.3 Optimization of the number of extraction steps

To ascertain the optimal number of extraction steps one kg soil is sieved (fraction < 2 mm), placed into a 4 L PP-bottle, and 610 ng 2,3-DCPA (in 5 mL aqueous solution) are added as internal standard. The slurry is shaken in an overhead shaker with 1.5 L aqueous 0.5 % Na_2CO_3 solution for 2 hours. After sedimentation the supernatant is removed, another 1.5 L aqueous 0.5 % Na_2CO_3 solution are added, and the mixture is shaken again for 2 hours. Then the supernatant is removed after sedimentation, one liter aqueous 0.5 % Na_2CO_3 solution is added, and the mixture is shaken again for 2 hours. All supernatants, obtained after the first, the second, and the third extraction, are treated separately. They are filtered through glass filters, transferred into several 250 mL PP-bottles, centrifuged twice at 15 °C with a speed of 10,000 rotations per minute for 6 minutes, and each of them is concentrated to an end volume of 0.5 L under reduced pressure (40 °C, 90 mbar). Three aliquots of 10 mL of each supernatant are acidified with H_2SO_4 (1 mol/L) to pH 5 and thereafter worked up as described in section 6.4.

6.6 Calculation of Kovats index

The soil matrix is very complicated and contains substances such as fulvic acids which disturb the determination of TCA and which are still present in the extract despite many extraction steps. Therefore, great care has to be taken to determine the retention time of TCA in order to separate it in pure form. To this end, the Kovats retention index is used.

The gas chromatography/flame ionization detection (GC/FID) is used to determine retention times of TCA-OMe and n-paraffins. Standard solutions of 10.7 mg/L commercial TCA-OMe in Et_2O and 13.0 mg/L commercial n-paraffins C9 - C12 are mixed in a ratio of 1:1 and 100 µL of this solution are injected onto the column. Additionally, 100 µL of TCA-OMe solution are injected to determine the retention time of this compound. The analytical conditions are given in table 4.

Gas chromatography	
Pre-column	0.5 m x 0.53 mm deactivated fused silica capillary (Varian-Chrompack)
Carrier gas	helium 4.6
Column flow rate	5.3 mL min^{-1}
Split flow rate	45 mL min^{-1}
Column, stationary phase	95 %-methyl -5 %-phenyl-polysiloxane 1.5 μm film thickness (CP-Sil 8 CB, Varian-Chrompack)
Column, dimensions	60 m x 0.53 mm
Temperature program	80 °C, 70 min isotherm

Tab. 4: Gas chromatographic conditions for Kovats index determination.

In order to determine the dead time of the column, 100 μL of methane are injected applying the conditions given above.

6.6.1 Soil sample preparation

One kg soil is sieved (fraction < 2 mm), placed into a 4 L PP-bottle, and shaken with 1.5 L aqueous 0.5 % Na_2CO_3 solution in an overhead shaker for 2 hours. After sedimentation the supernatant is removed, another liter aqueous 0.5 % Na_2CO_3 solution is added, and the mixture is shaken again for 2 hours. The aqueous phases are transferred into several 250 mL PP-bottles, centrifuged twice at 15 °C with a speed of 10,000 rotations per minute for 6 minutes, and concentrated to a volume of 0.5 L under reduced pressure (40 °C, 90 mbar).

50 g NaCl are added, then the sample is washed twice with 50 mL n-hexane and twice with 50 mL Et_2O, acidified with concentrated H_2SO_4 under cooling to pH 0.5, and extracted 3 times with 60 mL Et_2O. The combined organic layers are concentrated to 50 mL (room temperature, 360 mbar), extracted 3 times with 10 mL aqueous Na_2CO_3 solution (1 mol/L), and 2 times with 10 mL deionized water. The aqueous phase is washed 3 times with 20 mL Et_2O after acidification with concentrated H_2SO_4 to pH 5. Then it is further acidified to pH 0.5 and extracted 9 times with

20 mL Et_2O. The organic extract is dried by adding 25 g anhydrous Na_2SO_4, concentrated to a volume of 3 mL, dried again with 1 g anhydrous Na_2SO_4 and TCA is methylated with freshly prepared CH_2N_2 at 0 °C (ice cooling). CH_2N_2 is obtained by hydrolytic cleavage of 2.7 g N-nitroso-N-methyl-4-toluenesulfonamide with 1 mL 5 % KOH in a methanolic solution. After 12 hours, 10 mg of silica gel are added to quench the excess of CH_2N_2. After methylation the extract is diluted to an end volume of 5 mL and spiked 10:1 with a solution of 10.7 mg/L commercial TCA-OMe in Et_2O and 13.0 mg/L commercial n-paraffins C9 - C12 mixed in a ratio of 1:1. 100 µL of this solution are injected into the GC using conditions given in table 4.

6.7 Identification of co-eluting compounds

Due to the complexity of the soil matrix, the compounds which co-elute with TCA-OMe during chromatographic separation must be identified. For this purpose a soil sample is prepared (section 6.6.1) and analyzed by GC/MS applying the scan mode as well as selected ion monitoring mode. The conditions are given in table 5.

Gas chromatography	
Pre-column	0.5 m x 0.53 mm deactivated fused silica capillary (Varian-Chrompack)
Column, stationary phase	95 %-methyl -5 %-phenyl-polysiloxane 0.25 μm film thickness (CP-Sil 8 CB-MS, Varian-Chrompack)
Column, dimensions	15 m x 0.25 mm
Carrier gas	Helium 4.6; head pressure 70 kPa
Injection / sample aliquot	on column; 30 °C; 1.0 μL
Temperature program	30 °C, 3 min isotherm; 3 °C min^{-1} to 100 °C; 10 °C min^{-1} to 240 °C; 10 min isotherm
Interface temperature	240 °C
Mass spectrometry	
Ionization mode	electron-impact (EI)
Electron energy, anode current	230 eV, 70 μA
SEV-voltage	2000 - 2400 V
Ion source temperature	200 °C
Detection mode Selected-ion monitoring (SIM) Total-ion current (TIC)	m/z 117, 119 (TCA-OMe)

Tab. 5: Gas chromatographic and mass spectrometric conditions for identification of co-eluting compounds.

6.8 Isolation of TCA from soil

6.8.1 Soil sample preparation

One kg soil is sieved (fraction < 2 mm), placed into a 4 L PP-bottle, and shaken with 1.5 L aqueous 0.5 % Na_2CO_3 solution in an overhead shaker for 2 hours. After sedimentation the supernatant is removed, another liter aqueous 0.5 % Na_2CO_3 solution is added, and the mixture is shaken again for 2 hours. The aqueous phases

are transferred into several 250 mL PP-bottles, centrifuged twice at 15 °C with a speed of 10,000 rotations per minute for 6 minutes, and concentrated under reduced pressure (40 °C, 90 mbar). This procedure is repeated 109 times. Soil extracts are collected and combined.

For each kg soil, 2.5 L extraction agent are used, so from 109 kg soil corresponding to 27 soil bags each containing ca. 4 kg, a total volume of 272.5 L extract is obtained. The extract acquired at a time from 4 kg soil is concentrated to an end volume of 1 L under reduced pressure (40 °C, 90 mbar). To ensure the quality of the sample preparation procedure, TCA concentrations are monitored both after acidic extraction with MTBE and derivatization with PFPDE by GC/NCI/MS. Aliquots of 10 mL are taken of each concentrated extract (1 L) and analyzed as described in section 6.4.

The combined aqueous extracts are further concentrated under reduced pressure (40 °C, 90 mbar) to three sub-samples each of an end volume of 1 L and centrifuged at 15 °C with a speed of 10,000 rotations per minute for 6 minutes. To each sub-sample (pH 11 after concentration), 50 g NaCl are added. Then each sample is washed twice with 100 mL n-hexane to remove hydrocarbons and waxes and twice with 100 mL Et_2O to remove lipids, acidified with concentrated H_2SO_4 under cooling to pH 5, and washed again twice with 100 mL Et_2O to remove fatty acids, humic acids and phenols. Organic phases are discarded; aqueous phases are further acidified with concentrated H_2SO_4 to pH 0.5 and extracted 4 times with 100 mL Et_2O. Aqueous residues are discarded; the organic layers are concentrated to 50 mL (room temperature, 360 mbar), extracted 5 times with 10 mL aqueous Na_2CO_3 solution (1 mol/L) and 5 times with 10 mL deionized water. The aqueous phases are washed 5 times with 20 mL Et_2O after acidification with concentrated H_2SO_4 to pH 5 to remove the remaining fatty acids, humic acids and phenols. Then they are further acidified to pH 0.5 and extracted 9 times with 20 mL Et_2O. The aqueous residues are discarded; the organic extracts are dried by adding 25 g anhydrous Na_2SO_4, concentrated to a volume of 6 mL, dried again with 1 g anhydrous Na_2SO_4 and TCA is methylated with freshly prepared CH_2N_2 at 0 °C (ice cooling) as described in section 6.6.1. (figure 20). After methylation the volumes of extracts is reduced to 3 mL.

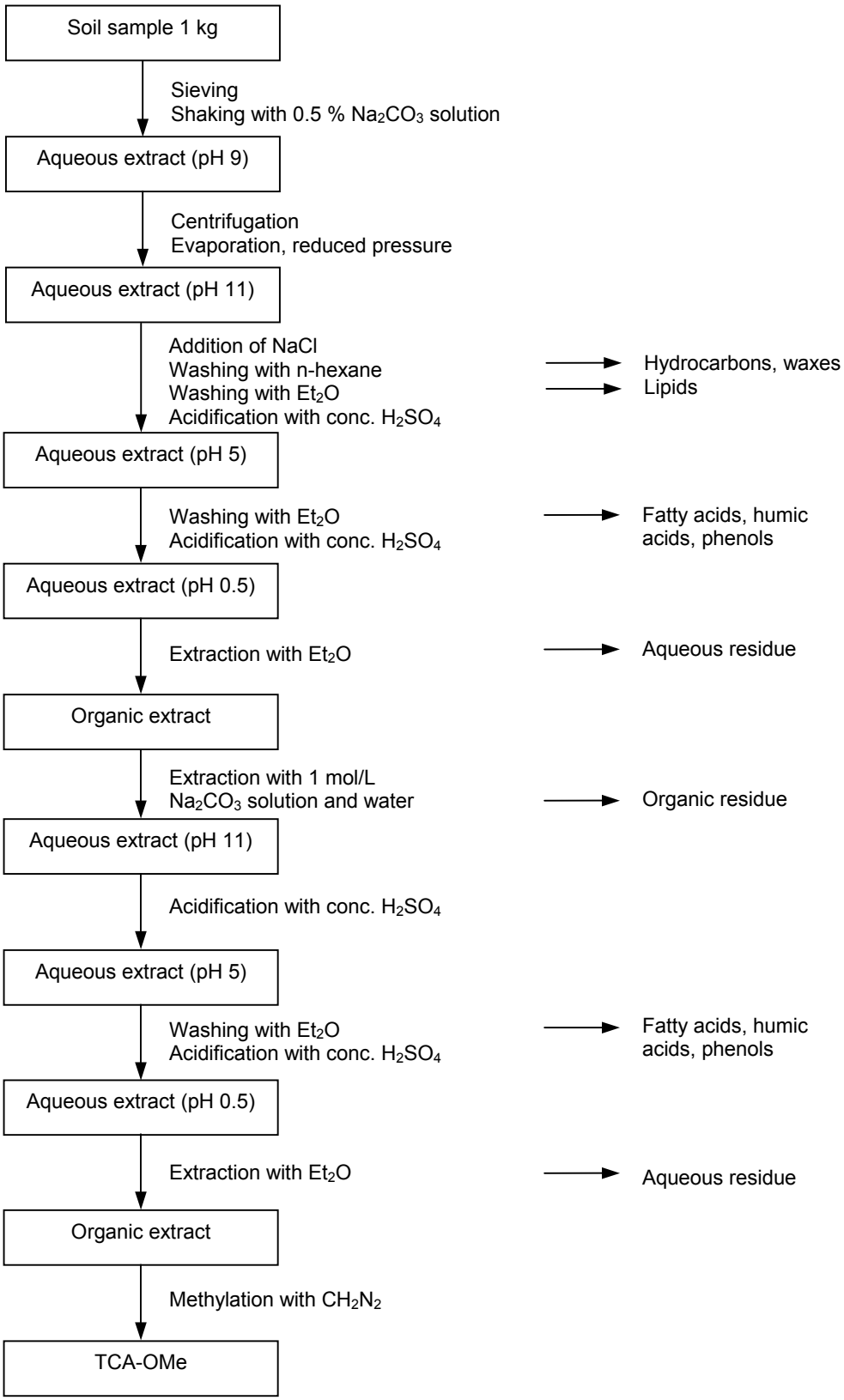

Fig. 20: Sample preparation for preparative gas chromatography.

Additionally, the water content (50 g soil are dried at 105 °C until constant mass is reached) and pH values (in aqueous 0.01 mol/L $CaCl_2$ solution) of sieved soil are determined in each bag.

6.8.2 Blank sample preparation

A blank sample is prepared from 272.5 L aqueous 0.5 % Na_2CO_3 solution. This solution is concentrated to 1 L under reduced pressure (40 °C, 90 mbar) and treated further as a sub-sample (see section 6.8.1).

6.9 Preparative capillary-gas chromatography (PC-GC)

To purify TCA for AMS-analysis PC-GC is applied. To this end, TCA-OMe is used which features the needed volatility but has a minimum of additional carbon atoms (Hasan, 2002). The introduction of a methyl group reduces the expected ^{14}C-isotope abundance to 66.7 PMC assuming that TCA originates fully from biomass.

6.9.1 PC-GC separation

To obtain pure TCA-OMe from soil samples, two-dimensional gas chromatographic separation on two columns coated with stationary phases of complementary polarity is applied. Initially, the order of columns used is determined by injecting a diluted aliquot of several mL of the soil extract sample.

In the first dimension, an apolar column (coated with 1.5 μm d_f 95 %-methyl-5 %-phenyl-polysiloxane, CP-Sil 8 CB, 60 m x 0.53 mm i.d.) is used. An aliquot of 75 μL of soil extract is injected. The fraction is collected in a glass U-trap cooled with liquid nitrogen, diluted in Et_2O, and re-injected on a semi-polar column (coated with 1.0 μm d_f 50 %-methyl-50 %-phenyl-polysiloxane, CP-Sil 24 CB, 30 m x 0.53 mm i.d.).

Also the opposite order of columns is tested, so that a semi-polar column is used in the first dimension. The fraction is collected in a glass U-trap cooled with liquid nitrogen, diluted in Et_2O, and re-injected on an apolar column.

The best separation of TCA-OMe from co-eluting compounds is achieved with an apolar column in the first dimension and a semi-polar one in the second dimension. The final conditions for analysis are given in table 6.

Gas chromatography	
Pre-column	0.5 m x 0.53 mm deactivated fused silica capillary (Varian-Chrompack)
Carrier gas	helium 4.6
Column flow rate	5.3 mL min^{-1}
Split flow rate	45 mL min^{-1}
First dimension	
Column, stationary phase	95 %-methyl -5 %-phenyl-polysiloxane 1.5 μm film thickness (CP-Sil 8 CB, Varian-Chrompack)
Column, dimensions	60 m x 0.53 mm
Temperature program	40 °C, 2.3 min isotherm; 7 °C min^{-1} to 260 °C; 45 min isotherm
Second dimension	
Column, stationary phase	50 %-methyl -50 %-phenyl-polysiloxane 1.0 μm film thickness (CP-Sil 24 CB, Varian-Chrompack)
Column, dimensions	30 m x 0.53 mm
Temperature program	40 °C, 2.3 min isotherm; 10 °C min^{-1} to 240 °C; 26 min isotherm
Cold injection system	
Injection speed	100 μL min^{-1}
Split mode	solvent venting with stop flow
Splitless time	1.5 min
Purge time	0.8 min
CIS program	0 °C, 0.8 min isotherm; 12 °C s^{-1} to 260 °C; 3 min isotherm
Preparative fraction collector	
Temperature of transfer line	200 °C
Temperature of switching device	210 °C
Temperature of waste trap	-30 °C
Temperature of sample trap	-80 °C

Tab. 6: Gas chromatographic conditions for preparative separation.

6.9.2 Purification of TCA in soil extracts

Over 250 injections of 75 μL soil extract each are performed. Over a time period of 1 minute (22.5 to 23.5 minutes) the fractions are collected in a glass U-trap cooled with liquid nitrogen, diluted in 3.5 mL Et_2O, and re-injected (28 injections, 125 μL) on a semi-polar column. Thus, the co-eluting compounds can be separated. For blank sample separation 8 injections of 75 μL are performed, diluted in 0.5 mL Et_2O, and re-injected 4 times (125 μL in each injection) on a semi-polar column.

6.10 Sample preparation for AMS

For collecting pure TCA-OMe separated during the second stage of preparative chromatography (see section 5.9.2), modified quartz U-traps (4 mm o.d./ 2 mm i.d.) filled with 0.01 g Ag and 0.3 g CuO are used (figure 21). Before collection, the GC oven temperature is set to 240 °C and the hot helium stream passes through the U-trap for 2 minutes to remove air and CO_2 adsorbed on the inner walls. During compound collection, the U-trap is connected to a preparative fraction collector and cooled with liquid nitrogen to -80 °C. Afterwards, the first constriction of the longer arm and the one of the vertical arm are sealed with a welding set and the sample is combusted in a muffle oven. The temperature is increased from room temperature to 900 °C at a rate of 7.5 °C per minute and then kept at 900 °C for 3 hours.

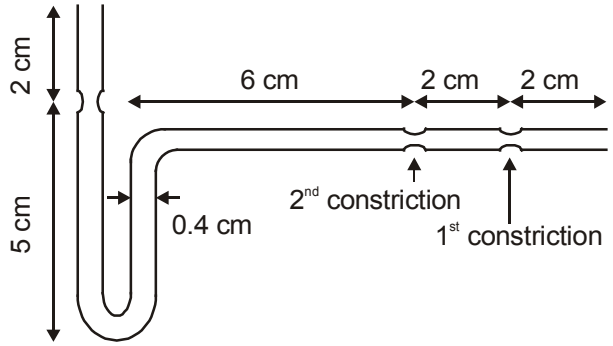

Fig. 21: A modified quartz U-trap with one constriction on the shorter arm and two constrictions on the longer one.

The CO_2 formed during combustion is recovered in a high vacuum system shown in figure 22. The trap is scored at the second constriction of the longer arm and joined to the sample trap cracker (figure 23) which is connected to the vacuum system. Valve V1 is opened and the system evacuated (10^{-3} mbar) with all valves open. After closing valve V3 to collect H_2O in trap T1, cooled with a dry ice/acetone mixture, and CO_2 in trap T2, cooled with liquid nitrogen, the second constriction of the sample is cracked and CO_2 originating from the sample trap is transferred to trap T2 within 20 minutes. Then valve V1 is closed and valve V3 opened so that non-condensible gases can be removed out of the system. After closing valve V4 and moving the liquid nitrogen bath from trap T2 to trap T4 and the dry ice/acetone bath from trap T1 to trap T2 to retain any H_2O that may have passed through trap T1 CO_2 is transferred from trap T2 to trap T4. Valve V3 is closed and the liquid nitrogen bath removed to allow trap T4 to reach room temperature in order to measure CO_2 pressure with an electronic manometer. Afterwards trap T4 is cooled again with liquid nitrogen for about 15 minutes, valve V6 is closed, and trap T4 demounted from the vacuum system (Hasan, 2002).

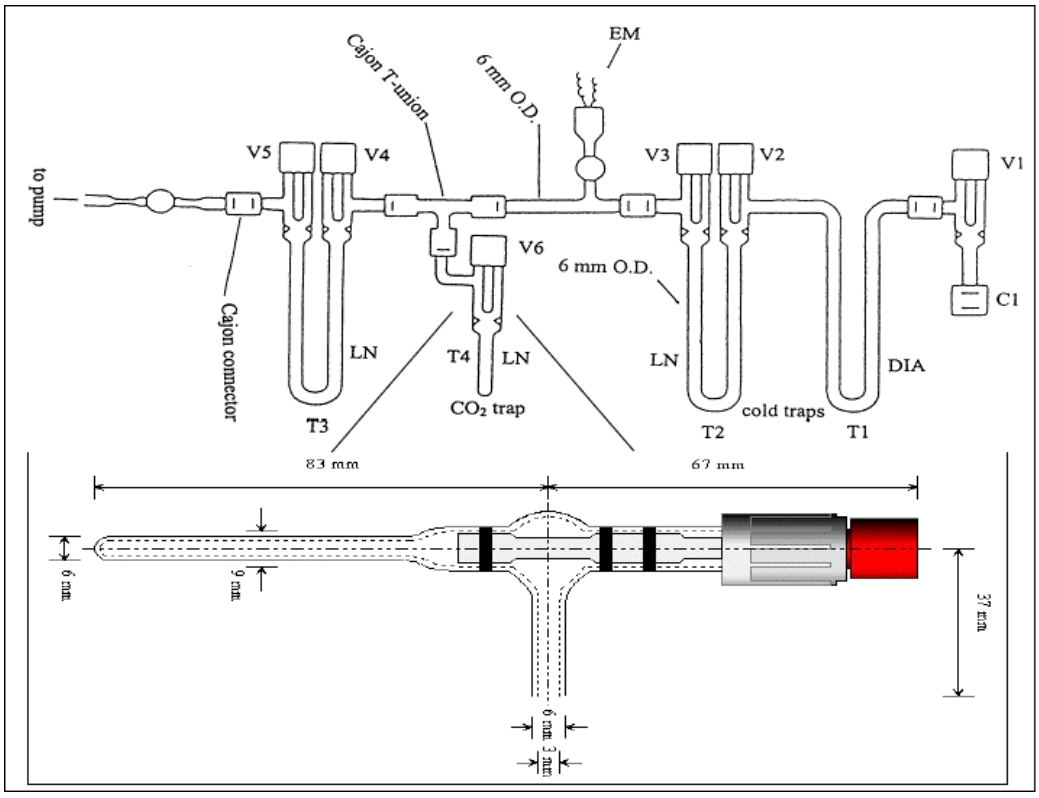

Fig. 22: High vacuum system used for CO_2 recovery and measurement after combustion: C1 - samlpe trap cracker, DIA - dry ice/acetone slurry, EM - electronic manometer, LN - liquid nitrogen, T1-T3: traps, T4: gas-tight cold-finger, V1-V6: valves (Hasan, 2002).

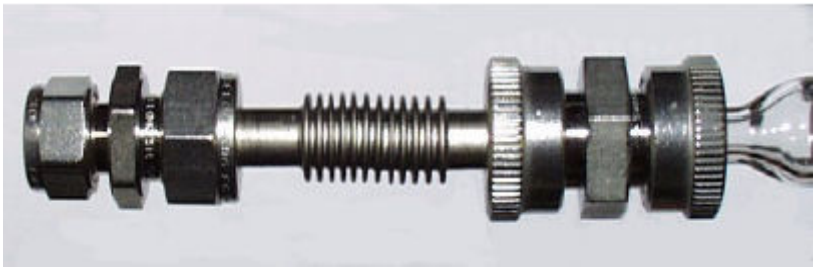

Fig. 23: Sample trap cracker (Hasan, 2002).

The CO_2 is transported from the gas-tight cold-finger to the AMS system as described in section 4.3.3.2. All sample preparation steps after sample combustion, i.e. CO_2 transfer to a high vacuum system, determination of its quantity, and AMS measurement, are performed at the Physics Institute at the University of Erlangen-Nürnberg.

7. Results and discussion

7.1 Quality assurance

7.1.1 Limit of detection, limit of quantification, precision

For quantification with GC/MS the limit of detection is defined as the sum of blank and triple standard deviation. The limit of quantification is defined as the sum of blank and six-fold standard deviation. For a blank value of 0.03 ± 0.01 μg/kg the detection limit is 0.06 μg/kg and the quantification limit 0.09 μg/kg. However, blanks vary from analysis to analysis, obtained values range between 0.03 and 0.3 μg/kg. The relative standard deviation of samples prepared simultaneously is in the range between 3 and 30 %. The problem of establishing the actual background levels in the laboratory as well as the level of contamination of all chemicals and glassware during TCA analysis has already been reported. Therefore, glass vials and PP-used bottles for sample preparation are cleaned as described in section 6.3 directly before use, in order to keep levels of blank samples low. For the same reason, sample preparation is performed in a clean-bench equipped with charcoal filters for air purification (Frank et al. 1989b; Frank et al. 1995).

7.1.2 Total error calculation

Total errors are calculated applying the error propagation method. For TCA quantification in soil errors of soil weighing and of internal standard solution preparation, three-fold dilution, and addition are considered resulting in a total error of 1.1 %.

For calibration, errors of internal standard solution preparation, three-fold dilution and addition, as well as TCA standard solution preparation, two-fold dilution and addition are taken into consideration giving a total error of 0.7 %.

In the case of the optimization of the soil extraction method for PC-GC analysis, an error of soil weighing, volumetric measurement of soil extract taken for analysis, as well as preparation, three-fold dilution and addition of internal standard are the components amounting to a total error of 1.2 %.

Internal standard addition method is used as an attempt to make corrections for uncontrollable random errors caused by other components during sample prepa-

ration procedures and transfer. The concentration of TCA is calculated from the ratio of peak areas of TCA and internal standard, not from the absolute TCA peak area.

7.1.3 Repeatability

The repeatability is defined as standard deviation of the value obtained after three injections of the same amount of sample within one day. In the case of GC/MS, it is 3 %. In that of PC-GC, repeatability is defined as standard deviation of the peak area of the same amount of standard injected five times within one day, for an apolar column (coated with 1.5 μm d_f 95 %-methyl-5 %-phenyl-polysiloxane, CP-Sil 8 CB, 60 m x 0.53 mm i.d.) it is 7 %, for a semi-polar one (coated with 1.0 μm d_f 50 %-methyl-50 %-phenyl-polysiloxane, CP-Sil 24 CB, 30 m x 0.53 mm i.d.) 5 %. As the repeatability of retention time is of great importance it is also determined for both columns during preparative separation. For the apolar one it is calculated to be 0.04 % (n = 5), for the semi-polar one 0.07 % (n = 5).

7.1.4 Sample trapping efficiency

For a semi-polar column coated with 1.0 μm d_f 50 %-methyl-50 %-phenyl-polysiloxane, CP-Sil 24 CB, 30 m x 0.53 mm i.d., a calibration curve is made to determine the recovery of compounds collected in glass U-traps. Sample volumes (standard solution of 117 mg/L commercial TCA-OMe in Et_2O) of 25, 50, 75, 100, 125, 150, and 175 μL are injected three times each.

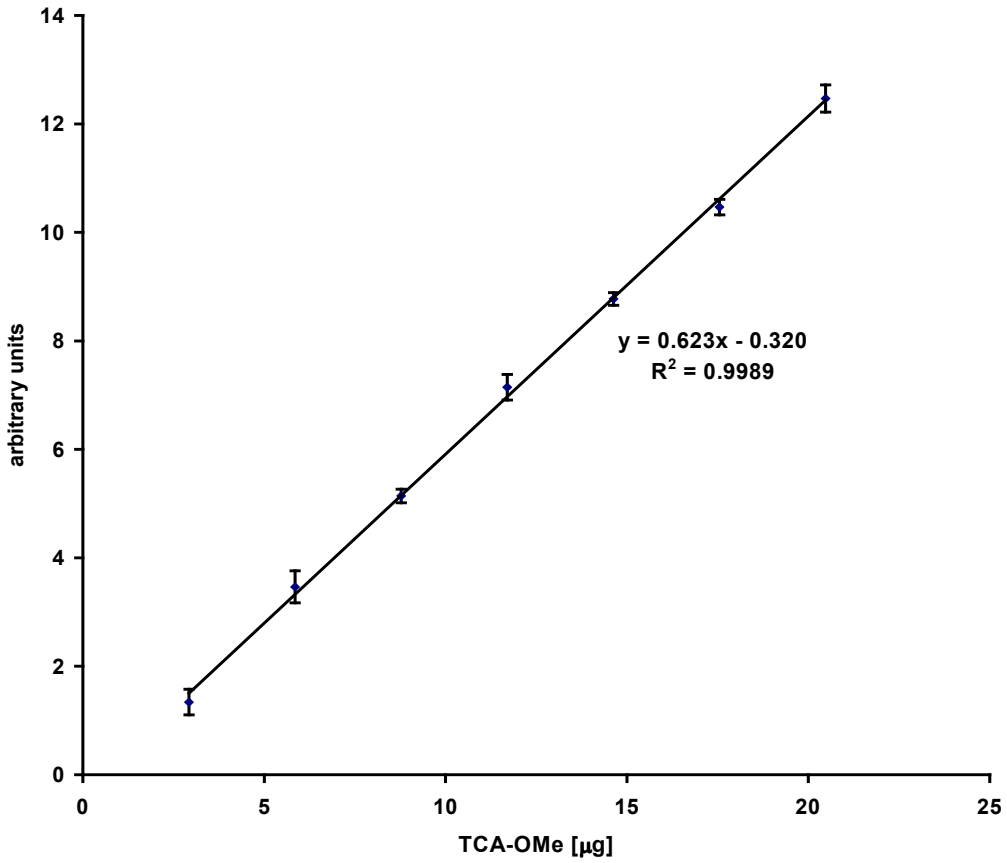

Fig. 24: Calibration curve for TCA-OMe on a semi-polar column coated with 1.0 µm d_f 50 %-methyl-50 %-phenyl-polysiloxane, CP-Sil 24 CB, 30 m x 0.53 mm i.d.; 40 °C, 2.3 min isotherm; 10 °C min^{-1} to 240 °C; 26 min isotherm.

To determine the recovery of the PC-GC separation 3 times 100 µL (11.7 µg TCA-OMe per injection) TCA-OMe solution (117 mg/L in Et_2O) are injected onto the apolar column and collected in an U-trap cooled to -80 °C. The collected amount (theoretically 35.1 µg) is diluted in 300 µL Et_2O, and 100 µL of this solution (11.7 µg) are injected on the semi-polar column, collected, and diluted in 200 µL Et_2O. For quantification 100 µL of this solution (5.8 µg) are injected again on the semi-polar column.

The recovered amount is 3.2 ± 0.5 µg (n = 5), corresponding to 55 % of the theoretical value. Such a low recovery is also described for fluorene in the literature for which different trapping temperatures were tested resulting in a recovery around 50 % at -16 °C and 0 °C and one as low as 40 % at 30 °C (Mandalakis et al., 2003). Thus, the trapping temperature does not seem to be the critical parameter for recovery. One of the factors giving rise to such a low recovery can be an incomplete sam-

ple transfer from vial to injection system, as the autosampler syringe needle does not sample the whole solution from a vial. Moreover, Et_2O a very volatile solvent evaporates during sample handling increasing the analyte concentration of the sample residue in a vial and so making losses bigger.

7.1.5 Combustion efficiency

To determine the combustion efficiency 6 times 100 µL TCA-OMe solution (117 mg/L in Et_2O) are injected onto the semi-polar column (conditions are given in table 6) and collected in modified quartz U-traps filled with 0.01 g Ag and 0.3 g CuO and cooled with liquid nitrogen to -80 °C. The traps are sealed and the samples are combusted in a muffle oven. Thereby, 3 samples are combusted by increasing the temperature from room temperature to 900 °C at a rate of 7.5 °C per minute, then they are kept at 900 °C for 3 hours. In case of the other 3 samples, the temperature is increased from room temperature to 600 °C at a rate of 5 °C per minute and is kept at 600 °C for 3 hours. Later on, CO_2 is evacuated from the traps in a high vacuum system and its quantity determined according to equation 5.3. The quartz U-trap of a sample combusted at 600 °C and one of a sample combusted at 900 °C were damaged in the process, so the recovery calculations are made for 2 samples at each temperature (table 7).

Sample	Temperature [°C]	Recovery [%]
1	600	46
2	600	50
3	900	78
4	900	98

Tab. 7: Sample recoveries applying a combustion temperature of 600 °C and 900 °C, respectively.

Sample combustion at 900 °C results in higher recoveries than that at 600 °C, so 900 °C is employed for real sample combustion.

7.2 Quantitative determination of TCA in soil

Water content and pH are determined in soil samples from Oberthulba and Altmugel. The values are given in table 8 together with TCA concentrations.

Soil	Water content [%]	pH	TCA concentration [µg/kg dw] mean value, n = 3
Oberthulba 5 - 10 cm	22	3.3	2.6 ± 0.3
Oberthulba 10 - 20 cm	12	3.6	3.1 ± 0.1
Altmugel 10 - 20 cm	56	2.6	0.7 ± 0.1
Altmugel 20 - 30 cm	32	2.7	0.7 ± 0.1

Tab. 8: Water content, pH values, and TCA concentration in soil samples taken in Oberthulba and Altmugel on 5 June 2002 and 11 June 2002, respectively; n - number of analyses.

In figure 25, a representative chromatogram of a soil sample from Oberthulba with a TCA concentration of 2.6 µg/kg dw is shown.

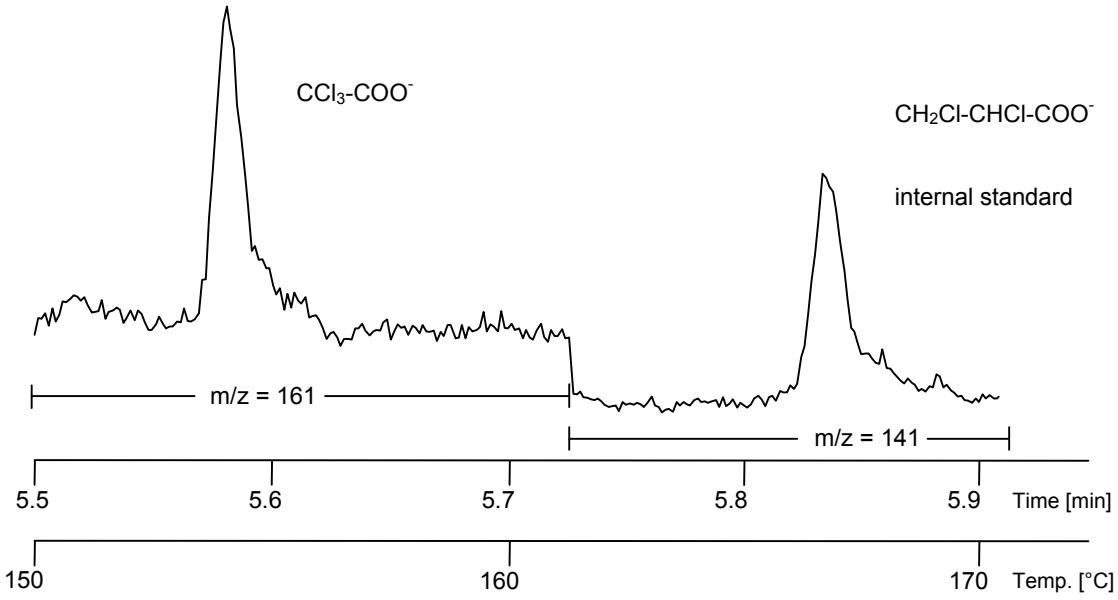

Fig. 25: GC/NCI/MS (SIM) chromatogram of a soil extract with a content of 2.6 pg TCA and 6.1 pg 2,3-DCPA in one injection; 60 °C, 1.5 min isotherm; 25 °C min^{-1} to 240 °C; 1.3 min isotherm.

7.3 Optimization of soil extraction process

7.3.1 Selection of extraction agent

Deionized water and aqueous 0.5 % Na_2CO_3 are compared as extraction agents for soil sample extraction and TCA is quantified with GC/NCI/MS. The TCA recovery employing aqueous 0.5 % Na_2CO_3 amounts to 0.6 ± 0.1 μg/kg (n = 3) and is higher than that of deionized water (< 0.1 μg/kg), so the first is chosen for soil extraction.

7.3.2 Optimization of extraction time

Extraction times of 1, 2, and 3 hours are compared. The results of TCA quantification with GC/NCI/MS are shown in table 9.

Extraction time [h]	TCA concentration [µg/kg] mean value, n = 3
1	0.7 ± 0.2
2	1.1 ± 0.2
3	0.7 ± 0.1

Tab. 9: TCA concentrations in samples extracted for 1, 2, and 3 hours, respectively (n - number of analysis).

TCA concentrations in samples which are shaken for 2 hours are the highest ones. Therefore, 2 hours are chosen as extraction time for further analysis.

7.3.3 Optimization of the number of extraction steps

The content of TCA in soil extracts after consecutive extraction steps is monitored in order to optimize the number of extraction steps. The results are given in table 10.

Extraction step	TCA [ng] in extract from 20 g soil sample	% of TCA extracted
1. extraction	14.4	76
2. extraction	3.6	19
3. extraction	0.9	5

Tab. 10: TCA content obtained in consecutive extraction steps.

As 95 % of the total TCA content in soil are recovered in the first and second extraction step, the third step is omitted to shorten the total sample preparing procedure.

7.4 Calculation of Kovats index

In figure 26 a chromatogram of a TCA-OMe standard injected with the commercial n-paraffins C9-C12 is shown.

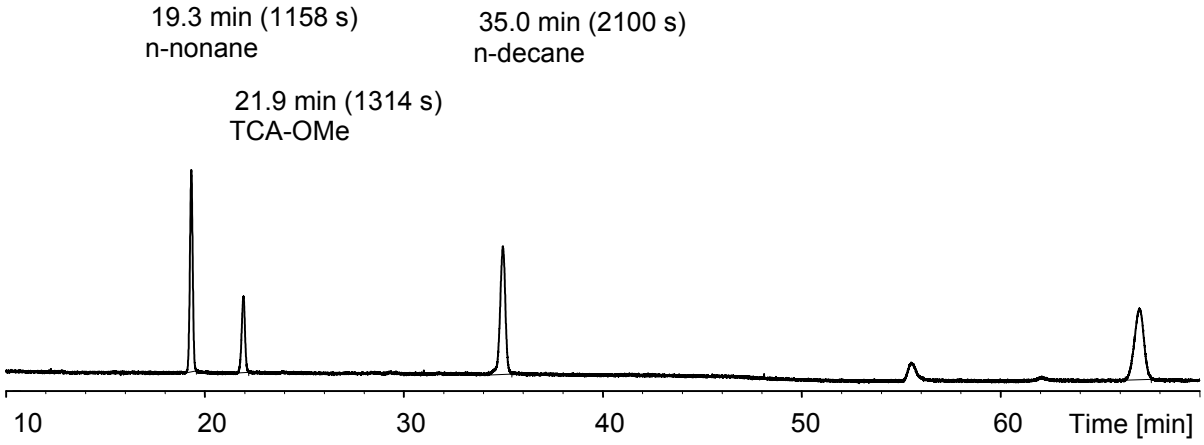

Fig. 26: Chromatogram of 100 μL of a standard solution of 10.7 mg/L commercial TCA-OMe in Et_2O and 13.0 mg/L commercial n-paraffins C9 - C12, mixed in a ratio 1:1 and separated on an apolar column coated with 1.5 μm d_f 95 %-methyl-5 %-phenyl-polysiloxane, CP-Sil 8 CB, 60 m x 0.53 mm i.d.; 80 °C, 70 min isotherm.

The dead time of the column after injection of 100 μL of methane is 5.2 min (312 s). Retention times of n-nonane, TCA-OMe and n-decane are given in table 11.

Compound	Retention time t_R [s]	Retention value $t'_R = t_R - t_0$	log (t'_R)
n-nonane	1158	846	2.93
TCA-OMe	1314	1002	3.00
n-decane	2100	1788	3.25

Tab. 11: Retention times, retention values and their logarithms of some n-paraffins and TCA-OMe; t_R - retention time, t'_R - retention value, t_0 - dead time of the column.

The Kovats index of TCA-OMe is calculated according to equation 5.1 as follows:

$$I_{TCA-OMe}(80°C) = 100\left(\frac{3-2.93}{3.25-2.93}\right)+100\cdot 9$$

$$I_{TCA-OMe}(80°C) = 922$$

Also the soil sample spiked with a solution of 10.7 mg/L commercial TCA-OMe in Et_2O and 13.0 mg/L commercial n-paraffins C9 - C12 mixed in a ratio of 1:1 is analyzed (figure 27).

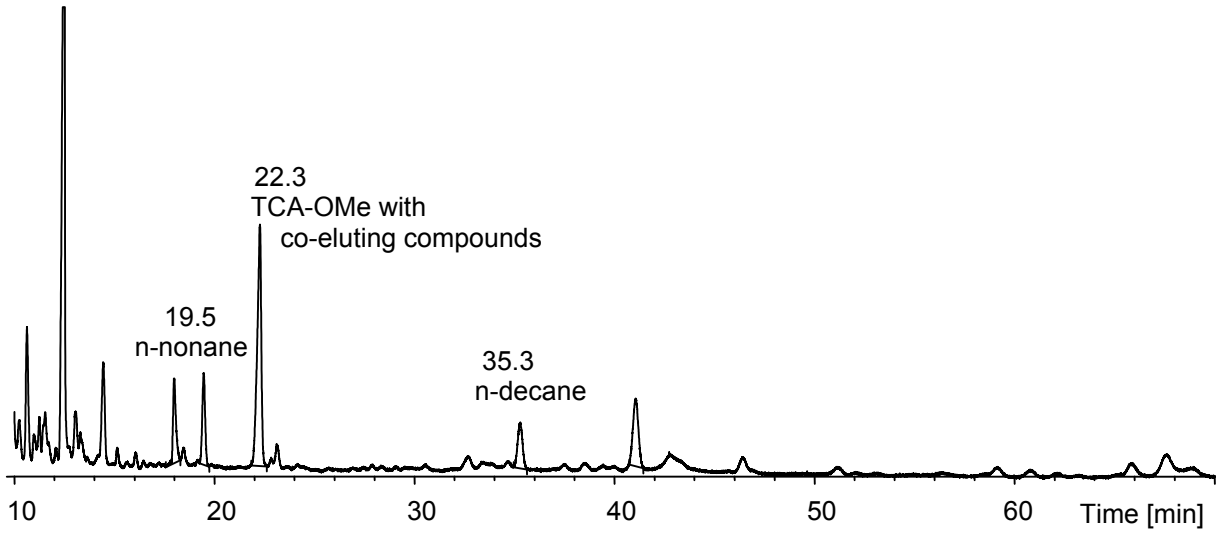

Fig. 27: Chromatogram of 100 µL soil sample mixed in a ratio 10:1 with a 1:1 solution of 10.7 mg/L commercial TCA-OMe in Et_2O and 13.0 mg/L commercial n-paraffins C9 - C12 separated on an apolar column coated with 1.5 µm d_f 95 %-methyl-5 %-phenyl-polysiloxane, CP-Sil 8 CB, 60 m x 0.53 mm i.d.; 80 °C, 70 min isotherm.

As there is at least one compound, possibly more, co-eluting with TCA-OMe identification of the major compund with GC/MS is performed.

7.5 Identification of co-eluting compounds

Compounds which co-elute with TCA-OMe during chromatographic separation are identified with GC/EI/MS in the TIC modus.

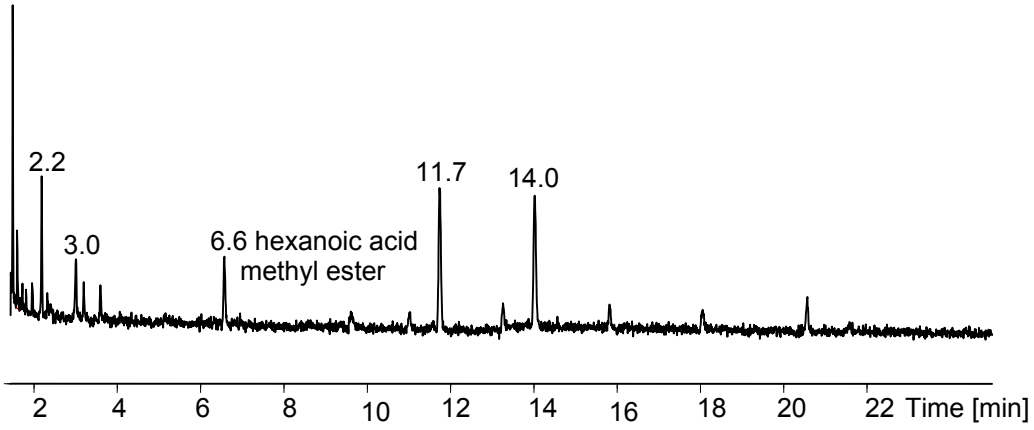

Fig. 28: GC/EI/MS (TIC) chromatogram of soil sample; 30 °C, 3 min isotherm; 3 °C min^{-1} to 100 °C; 10 °C min^{-1} to 240 °C; 10 min isotherm.

As in the TIC modus no TCA-OMe is detected (figure 28) the same sample is injected in the SIM modus and the TCA-OMe specific ions 117 and 119 are monitored (figure 29).

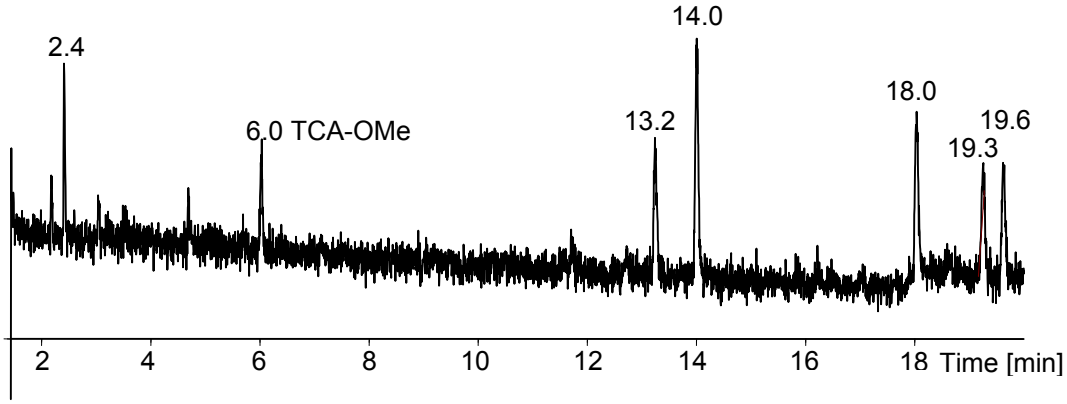

Fig. 29: GC/EI/MS (SIM) chromatogram of soil sample, ions with m/z ratio of 117 and 119 are monitored; 30 °C, 3 min isotherm; 3 °C min^{-1} to 100 °C; 10 °C min^{-1} to 240 °C; 10 min isotherm.

The presence of TCA-OMe is confirmed at a retention time of 6 minutes (figure 29). The library match of the peak eluting half a minute later than TCA-OMe (6.6 min, figure 28) proves it to be hexanoic acid methyl ester. The delay of its retention time comparing to the retention time of TCA-OMe can be explained by differ-

ences in analytical conditions used in PC-GC and GC/MS analysis. In the first case, the separation is isothermal, whereas in the latter, a temperature program is applied, thus improving the separation. Also column dimensions like inner diameter and film thickness can influence the retention of compounds.

7.6 Sample preparation for preparative isolation

TCA concentration in each extract derived from 4 kg soil sample (one bag) is monitored by analysis with GC/NCI/MS. The values given in table 12 are obtained by single analysis.

Bag number	pH	Water content [%]	TCA [µg/kg]	Bag number	pH	Water content [%]	TCA [µg/kg]
1	3.8	6	1.5	15	3.6	8	0.4
2	3.6	8	1.1	16	4.1	13	0.1
3	3.9	11	0.5	17	3.7	7	1.0
4	3.7	10	0.3	18	3.7	8	0.5
5	3.6	9	2.3	19	3.7	11	0.2
6	3.7	10	0.8	20	3.8	11	0.6
7	3.5	7	2.1	21	3.6	8	0.8
8	3.2	8	1.2	22	3.8	11	1.0
9	3.1	12	2.9	23	3.9	5	0.8
10	3.3	9	1.0	24	3.8	10	1.0
11	3.3	8	1.7	25	3.7	10	3.0
12	3.2	7	1.2	26	3.7	8	1.7
13	3.3	9	0.4	27	3.9	9	1.0
14	3.1	8	1.6				

Tab. 12: Water content, pH values, and TCA concentration in soil samples taken in Oberthulba on 25 July 2002.

The mean value of the TCA concentration in all soil samples shown in table 13 is 1.1 ± 0.8 µg/kg. Samples taken from a large area show a big variability of TCA concentration due to soil inhomogeneity.

7.7 Preparative capillary-gas chromatography

7.7.1 PC-GC separation

In figure 30 the chromatogram of a soil extract separated on an apolar column in the first dimension is shown.

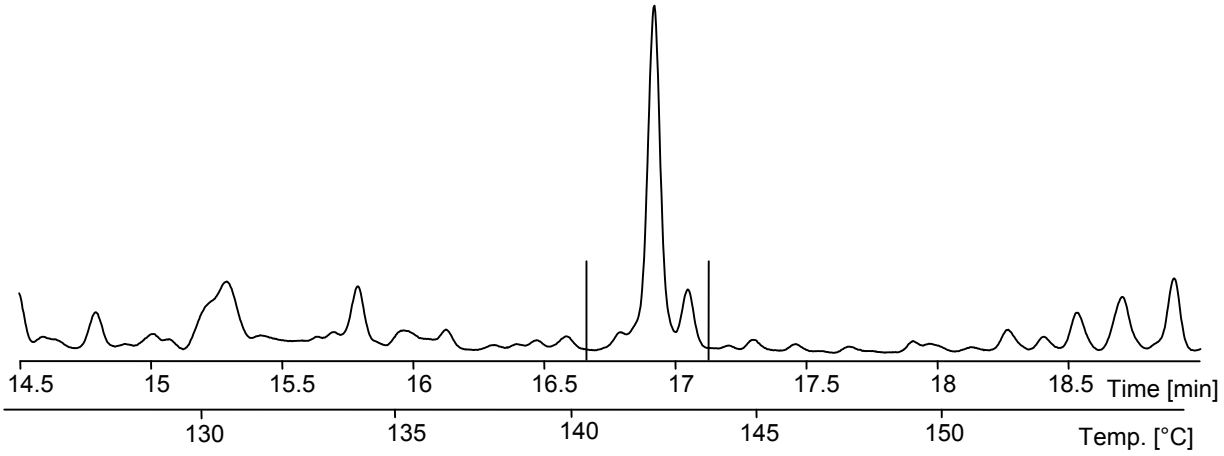

Fig. 30: Chromatogram of 75 µL of soil extract separated on a column coated with 1.5 µm d_f 95 %-methyl-5 %-phenyl-polysiloxane, CP-Sil 8 CB, 60 m x 0.53 mm i.d.; 40 °C, 2.3 min isotherm; 7 °C min^{-1} to 260 °C; 45 min isotherm; fraction between 16.6 and 17.2 minutes is collected for the second preparative separation (figure 31).

The chromatogram of a fraction containing TCA-OMe collected on an apolar column and re-injected on a semi-polar column is shown in figure 31.

-

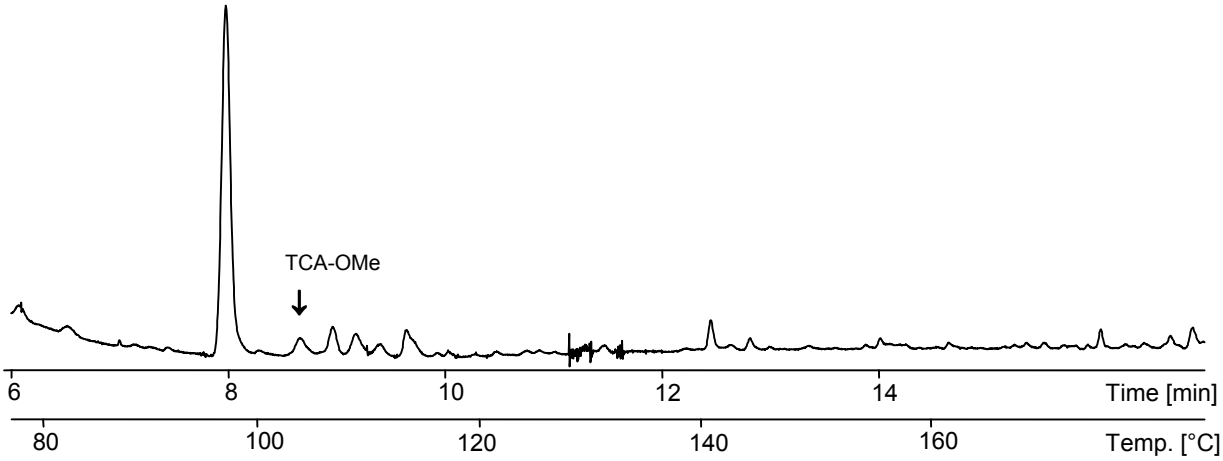

Fig. 31: Chromatogram of a fraction containing TCA-OMe collected on an apolar column, re-injected and separated on a semi-polar column coated with 1.0 μm d_f 50 %-methyl-50 %-phenyl-polysiloxane, CP-Sil 24 CB, 30 m x 0.53 mm i.d.; 40 °C, 2.3 min isotherm; 10 °C min^{-1} to 240 °C; 26 min isotherm.

Also the opposite order of columns using a semi-polar column in the first dimension is tested (figure 32). The TCA-OMe containing fraction is collected in a glass U-trap cooled with liquid nitrogen, diluted in Et_2O and re-injected on an apolar column (figure 33).

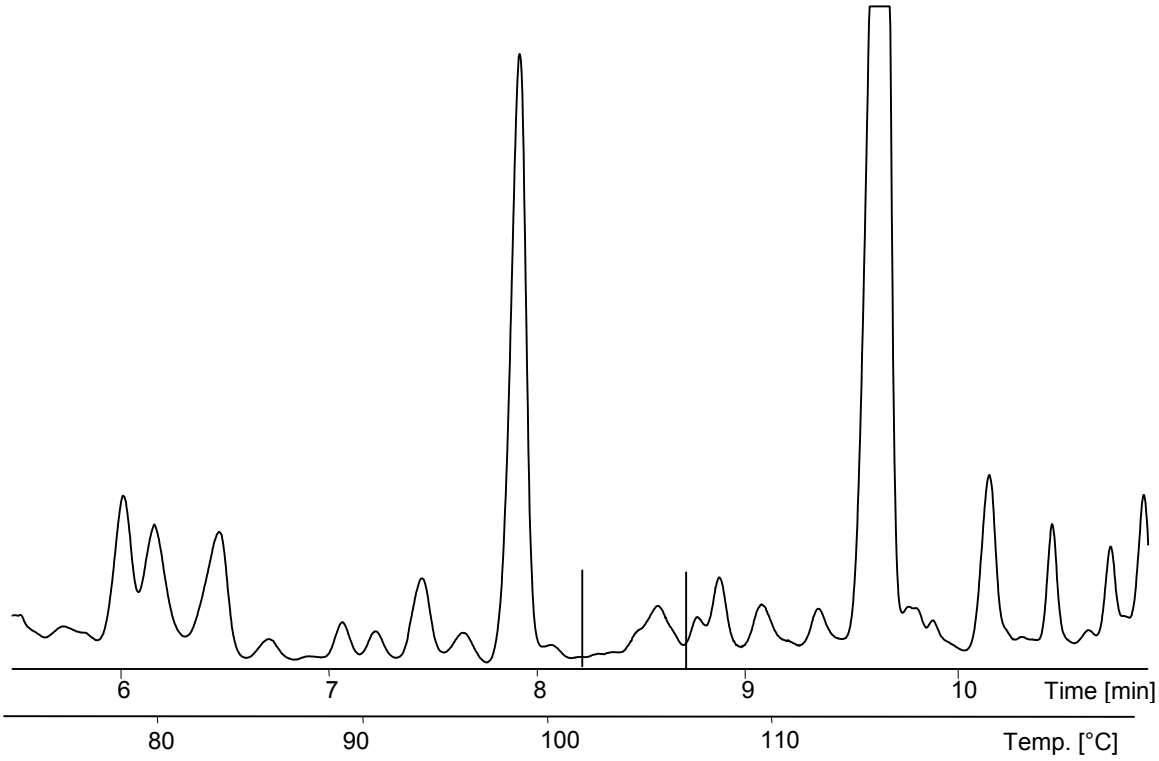

Fig. 32: Chromatogram of 75 µL of soil extract separated on a column coated with 1.0 µm d_f 50 %-methyl-50 %-phenyl-polysiloxane, CP-Sil 24 CB, 30 m x 0.53 mm i.d.; 40 °C, 2.3 min isotherm; 10 °C min^{-1} to 240 °C; 26 min isotherm; fraction between 8.2 and 8.7 minutes is collected for the second preparative separation (figure 33).

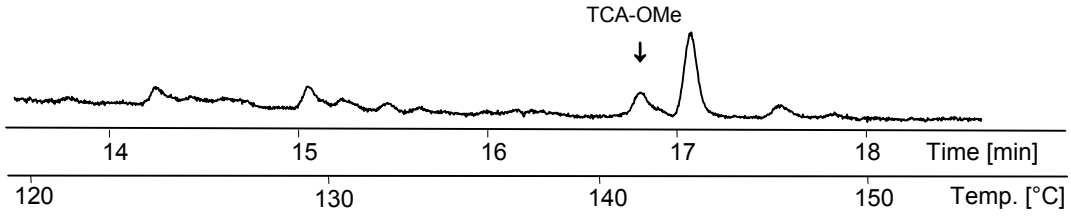

Fig. 33: Chromatogram of a fraction containing TCA-OMe collected on a semi-polar column, re-injected and separated on an apolar column coated with 1.5 µm df 95 %-methyl-5 %-phenyl-polysiloxane, CP-Sil 8 CB, 60 m x 0.53 mm i.d.; 40 °C, 2.3 min isotherm; 7 °C min^{-1} to 260 °C; 45 min isotherm.

7.7.2 Purification of TCA in soil extracts

Chromatograms depicting separation of real soil samples are given in figure 34. The extract is more concentrated than the one used for determining the final column order. As a consequence, the chromatograms look different.

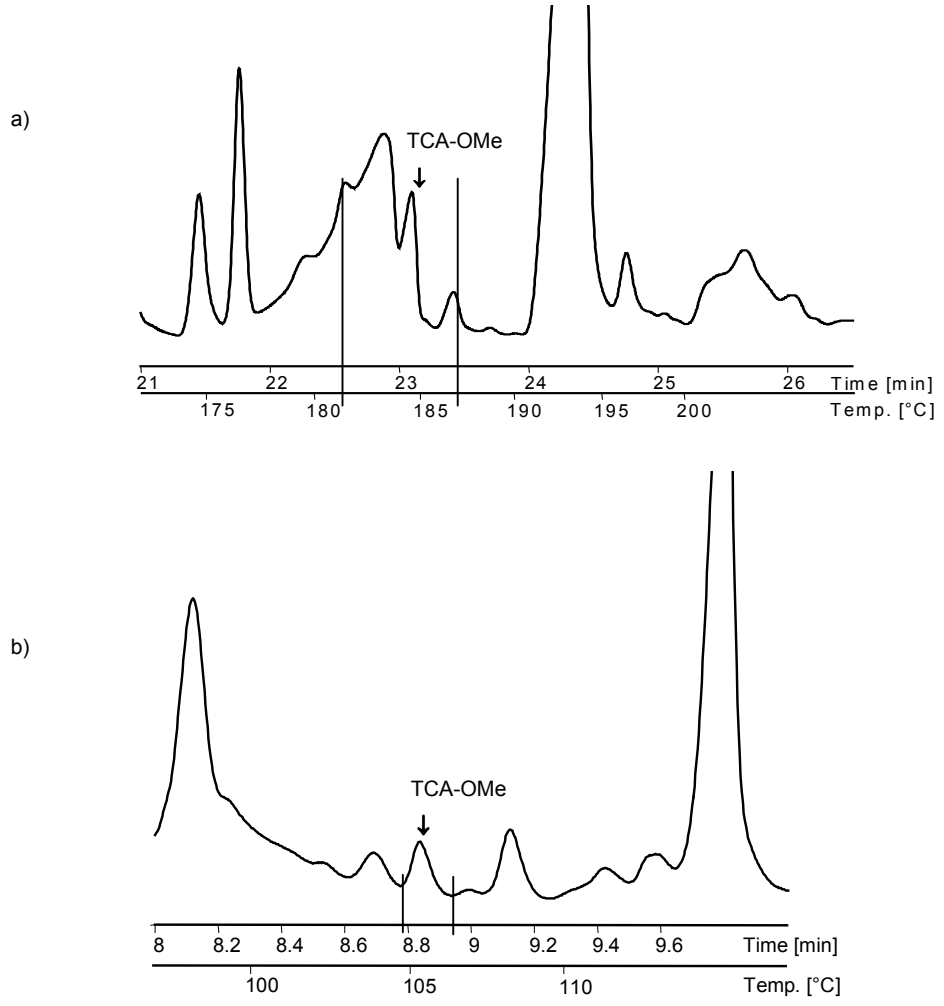

Fig. 34: a) Chromatogram of 75 µL soil extract injected on 95 %-methyl-5 %-phenyl-polysiloxane, 60 m x 0.53 mm i.d., 1.5 µm d_f; 40 °C, 2.3 min isotherm; 7 °C min^{-1} to 260 °C; 45 min isotherm; b) Chromatogram of 125 µL of a fraction separated on an apolar column, re-injected on 50 %-methyl-50 %-phenyl-polysiloxane, 30 m x 0.53 mm i.d., 1.0 µm d_f; 40 °C, 2.3 min isotherm; 10 °C min^{-1} to 240 °C; 26 min isotherm. In both chromatograms lines indicate the collected fractions.

Based on those chromatograms (figure 34) it is decided which fractions containing TCA-OMe should be collected. To this end, the valve switch of the PC-GC

must be triggered manually. This is necessary as the retention time of TCA-OMe varies by ± 1 minute thus rendering an automated peak recognition program useless.

Moreover, as not all the matrix compounds are removed during the sequential extraction of a soil sample, the chromatographic injection system is strongly contaminated with them. Despite a very long time (45 minutes) of column heating at 260 °C, it is impossible to avoid the shift in retention time which occurs with increasing number of injections. Therefore, it is necessary to clean the whole injection system and change the Tenax liner after a maximum of 8 chromatographic separations. The very good retention time repeatability obtained with standards is unattainable with real soil sample extracts due to strong soil matrix effects.

As Et_2O is used as solvent, concentrations of compounds in the autosampler vial increase with time as Et_2O evaporates. For this reason, additional cryostatic cooling of the autosampler tray must be applied.

The TCA-OMe amount obtained after preparative separation and calculated from the peak area is 10 μg which corresponds to 2 μg carbon. This is a much lower value than expected. The reasons are the following:

- loss of 10 mL from each of the twenty seven 1-L extracts for GC/MS analysis in order to control the TCA concentration,
- loss of analyte during multiple extraction steps,
- incomplete derivatization with diazomethane,
- loss of solution in autosampler vial, as the syringe does not draw it up completely,
- low trapping efficiency,
- losses caused by adjusting TCA-OMe retention time after each injector cleaning.

7.7.3 Blank sample

Analogous to the real soil sample a blank sample is prepared and separated first on an apolar column. The fraction in which TCA-OMe is expected is collected in a glass U-trap cooled with liquid nitrogen, diluted in Et_2O, and re-injected onto a semi-polar column (figure 35).

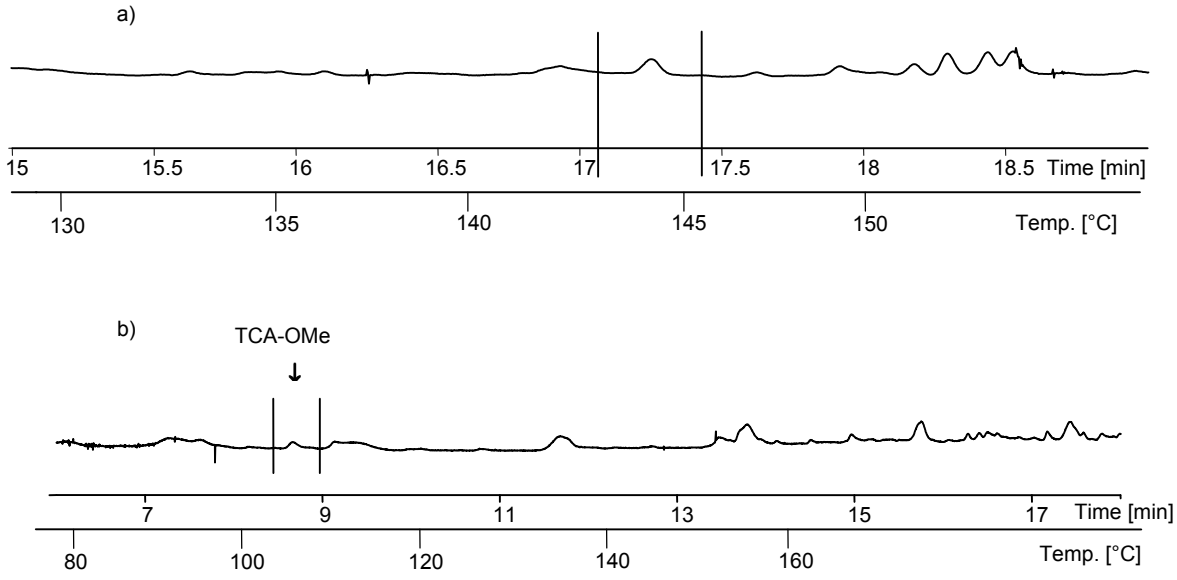

Fig. 35: a) Chromatogram of 75 µL blank sample injected on 95 %-methyl-5 %-phenyl-polysiloxane, 60 m x 0.53 mm i.d., 1.5 µm d_f; 40 °C, 2.3 min isotherm; 7 °C min^{-1} to 260 °C; 45 min isotherm; b) Chromatogram of 125 µL of a fraction separated on an apolar column, re-injected on 50 %-methyl-50 %-phenyl-polysiloxane, 30 m x 0.53 mm i.d., 1.0 µm d_f; 40 °C, 2.3 min isotherm; 10 °C min^{-1} to 240 °C; 26 min isotherm. In both chromatograms lines indicate the collected fractions.

7.8 AMS analysis

The amount of CO_2 recovered in a high vacuum system is calculated from its pressure value according to the following equation (Uhl, 2004):

$$m_c = 4.951\,mg\,\frac{p_{CO_2}\cdot V}{10^4\,mbar\cdot mL} \qquad (7.1.)$$

m_C: carbon mass [mg]

p_{CO2}: carbon dioxide pressure [mbar]

V: reduction vessel volume [mL]

The ^{12}C and ^{13}C ion abundances measured as electric current in Faraday cups as well as number of ^{14}C ions counted individually in a gas ionization detector are given in appendix 1.

To determine the spectrometer's blank values and possible contaminations deriving from sample preparation ^{14}C-free graphite samples of different masses are used. They undergo the same preparation procedure as actual samples, thereby they are contaminated with modern carbon, including ^{14}C. Consequently, the amount of ^{14}C in these samples is determined and represents a measure for the contamination with carbon. A calibration curve is generated (figure 36) with which the $^{14}C/^{13}C$ ratios of measured and calibration samples have to be corrected as both sample types are affected by this impurity. Due to the ^{14}C background this correction gives rise to large uncertainties, especially for carbon masses in the µg range as can be seen in figure 36. Therefore, the use of solid carbon targets in the ion source is given up in favor of the use of CO_2 in a gas ion source (see 4.3.3.2).

$$\left(\frac{^{14}C}{^{13}C}\right)_{Corr} = \left(\frac{^{14}C}{^{13}C}\right)_{Sample/S\tan dard} - \left(\frac{^{14}C}{^{13}C}\right)_{Graphite} \qquad (7.2.)$$

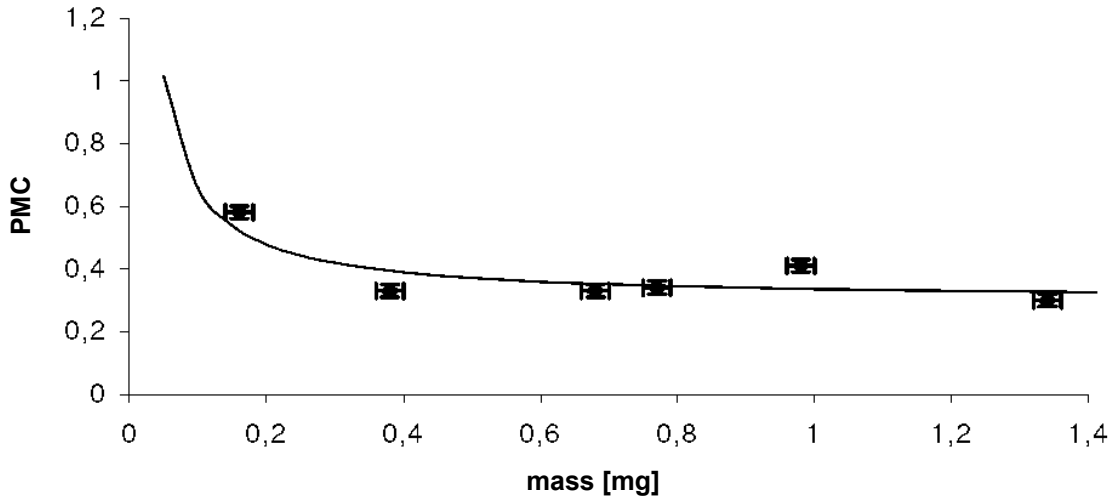

Fig. 36: PMC values for ^{14}C-free carbon depending on the sample mass (Uhl, 2004).

The carbon isotopes show an isotope fractionation effect: although ^{12}C, ^{13}C, and ^{14}C are chemically identical, they have different median speeds at the same temperature due to their different masses. Consequently, to the same extent they do not participate in physical processes, e.g. gas volume change or incomplete chemical

conversions. The isotope ratios are shifted in the direction of the lighter isotopes, that is why they vary differently but characteristically from the atmospheric ratio; e.g. in the CO_2 assimilation of plants the lighter carbon isotope is preferred. This leads to the enrichment of ^{12}C relative to ^{13}C, ^{12}C relative to ^{14}C, and ^{13}C relative to ^{14}C. Therefore, all isotope ratio measurements must be corrected. To this end, the ^{12}C and ^{13}C ratio is also considered which can only differ from the atmospheric value due to an isotopie effect. This deflection of the initial $^{13}C/^{12}C$ ratio in different samples of a defined standard value is given as $\delta^{13}C$-value.

$$\delta^{13}C_{Sample} = \frac{\left(\frac{^{13}C}{^{12}C}\right)_{Sample} - \left(\frac{^{13}C}{^{12}C}\right)_{PDB}}{\left(\frac{^{13}C}{^{12}C}\right)_{PDB}} \cdot 1000‰ \qquad (7.3.)$$

As a standard value, the $^{13}C/^{12}C$ ratio of PeeDee Belemnite (PDB; limestone) is used ($^{13}C/^{12}C_{PDB}$ = 0.0112372), $\delta^{13}C_{PDB}$ = 0 $^{o}/_{oo}$.

Most plants growing in moderate climate zones use the C3-photosynthesis cycle for CO_2 assimilation and have a $\delta^{13}C$ value of about -25 $^{o}/_{oo}$. In warmer regions also C4 plants are present which have a $\delta^{13}C$ value of about -13 $^{o}/_{oo}$. As most plants use the C3 cycle it is agreed to refer all radiocarbon measurement results to the reference value of $\delta^{13}C$ = -25 $^{o}/_{oo}$. Therefore, the determined $^{14}C/^{13}C$ values are corrected as follows:

$$\left(\frac{^{14}C}{^{13}C}\right)_{CorrSample} = \left(\frac{^{14}C}{^{13}C}\right)_{Sample} \left[1 - \left(\delta^{13}C_{Sample} + 0.025\right)\right] \qquad (7.4.)$$

$$\left(\frac{^{14}C}{^{13}C}\right)_{CorrS\,tan\,dard} = 0.746 \left(\frac{^{14}C}{^{13}C}\right)_{S\,tan\,dard} \left[1 - \left(\delta^{13}C_{S\,tan\,dard} + 0.025\right)\right] \qquad (7.5.)$$

The PMC value of the standard oxalic acid (Ox-II) is 134.06. Therefore, it is necessary to correct the standard values with the factor 0.746 = 1/1.3406. For the soil sample a typical $\delta^{13}C$ value of -26 $^{o}/_{oo}$ is given.

The PMC value is calculated after comparison of the corrected $^{14}C/^{13}C$ ratio for the sample and the corrected $^{14}C/^{13}C$ ratio for oxalic acid with the known PMC value of 134.06.

$$PMC = \frac{\left({}^{14}C/{}^{13}C\right)_{CorrSample}}{\left({}^{14}C/{}^{13}C\right)_{CorrS\,\mathrm{tan}\,dard}} \cdot 100\,\% \qquad (7.6.)$$

Moreover, as methylation with diazomethane adds an additional carbon atom, this must be taken into account and a proper correction must be made. The PMC value of TCA can be calculated from the known PMC value of TCA-OMe (Hasan, 2002):

$$PMC_E = PMC_A f_A + PMC_M f_M \qquad (7.7.)$$

PMC_E: PMC in TCA-OMe

PMC_A: PMC in TCA

PMC_M: PMC in methyl group added during methylation

f_A: fraction of carbon originating from TCA (2/3)

f_M: fraction of carbon originating from the introduced methyl group (1/3)

Being a synthetic chemical the PMC_M value of N-nitroso-N-methyl-4-toluenesulfonamide is 0. Therefore, the PMC value of TCA can be calculated directly from the PMC_E value:

$$PMC_A = 1.5\ PMC_E \qquad (7.8.)$$

In table 13, PMC values of calibration standards, ^{14}C-free graphite, as well as those of samples are given with a probability of 68 %. Weighted averages are given as the result of several injections of one sample. The number of runs depends on counting rates; at least 16 runs of 60 s for each sample are performed. Due to the Poisson-distribution, counting rates of 40,000 are required in order to obtain 0.5 % precision. For the soil sample, counting rates of 100 lead to a statistical precision of 10 %.

Sample name	Notes	PMC	PMC error [%]	$\delta^{13}C$	Mass [µg]
Graphite + Fe	solid	0.21	14	-	2000
Ox II	solid	133	0.8	-	2000
Ox 50 %	solid	49.6	0.8	-	2000
IAEA-C5	solid	22.6	1.1	-	2000
Graphite	gas	2.9	9	-	5
Ox 50 %	gas	47.5	2	-	2
Background	3 GC runs	1.4	28	-25	0.7
Soil sample	TCA-OMe	2.2	11	-26	1.2
Blank sample	-	4.8	10	-25	10
Ox II	solid	135	0.8	-	2000

Tab. 13: PMC values of calibration standards, ^{14}C-free graphite, and those of samples determined in the present thesis, all obtained by AMS measurement (Ox II - oxalic acid with PMC mean value of 134.06, Ox 50 % - oxalic acid with PMC value of 49.58, IAEA-C5 - International Atomic Energy Agency wood standard).

The mass of carbon obtained after combustion of the blank sample is almost ten-fold higher than the mass of the real sample. Most probably the reason for this is a difference in the technique used for sealing the samples. The blank sample prepared before the real one is sealed with a conventional brazing and welding set meaning that the tip of the flame is directed towards one point of the U-trap such causing uneven heating. As a consequence, a hole occurred in the sealing area and some gas combustion products enter the trap contaminating the blank sample and such increase the carbon mass. Therefore, the nozzle of the brazing and welding set is modified. It is constructed as a ring with four openings for the flame enabling an even heating of the quartz trap from four sides during the sealing process and thus reducing the likelihood of the formation of holes.

The PMC value of 2.2 for TCA-OMe isolated form soil samples taken in Oberthulba indicates that TCA there is of anthropogenic origin. The PMC value of TCA calculated according to equation 7.8 is 3.3 PMC. If TCA were of biogenic origin, a value of 66.7 PMC would be expected as one additional carbon from

a petrochemical source is introduced into the molecule during the derivatization of TCA to its methyl ester. The result proves that the TCA found in soil in Oberthulba is an atmospheric breakdown product of tetrachloroethylene and not a product of any soil microbial process. It denies the speculations about TCA formation in situ in soil and proofs that TCA formed in the atmosphere is deposited in soil with rain water and then it is either transported into deeper soil layers as it is very well water soluble or taken up through trees' roots.

The TCA mass balances, which are the main reasons for proposing natural formation of TCA, are very inaccurate as the uncertainties are enormous i.e. the large standard deviation of about 80 % (Schöler et al., 2003b). Also the experiments conducted in order to prove the natural formation of TCA do not allow to draw this conclusion. It is claimed that TCA can be formed through enzymatic conversion of short chained aliphatic acids like acetic acid in the presence of chloroperoxidase, sodium chloride and hydrogen peroxide (Haiber et al., 1996). However, fully chlorination of acetic acid to yield TCA is not straight forward; further chlorination of monochloroacetic acid obtained from acetic acid in the presence of bentonite at 130 °C results only in the formation of dichloroacetic acid yielding 1.4 % related to acetic acid (Hasan, 2002; Heisel, 1953).

It is speculated that the chloroperoxidase mediated chlorination of resorcinolic acid yields TCA at pH < 7, and chloroform at pH > 7. A strong positive correlation between the concentrations of TCA and chloroform in soil is found (Hoekstra et al., 1999b). The authors conclude that this correlation is the result of chloroperoxidase activity, and not the consequence of TCA decomposition yielding chloroform. However, taking the typical range of pH values for the soil top layer, i.e. 3 - 6, into consideration chloroform cannot be formed under those conditions, thus the only explanation of its presence in high amounts and its positive correlation to TCA concentration is the decomposition of TCA. Consequently, the conclusion of natural TCA and chloroform formation based on the aforementioned study cannot be made. Long time experiments are needed to observe changes in TCA and chloroform concentrations placing special emphasis on the fact that chloroform can be a decarboxylation product of TCA.

Also doping experiments aiming to explain the active turnover of TCA can not be taken as a clear evidence of TCA in-situ formation (de Winkel et al., 2004). Application of ^{37}Cl-spiked sodium chloride results in increased ^{37}Cl level in TCA

extracted from soil but this fact can be the result of a spontaneous halogen exchange reaction in organic molecules, known as the “Finkelstein reaction”, so TCA in-situ formation cannot be concluded from this experiment (Frank, 2004).

The TCA found in firn and glacier ice (von Sydow et al., 1999) also does not provide any proof for the theory that it can be naturally formed as the found TCA concentrations do not differ from the blank values.

In this regard, the radiocarbon method is the only possibility to give a clear answer leaving no doubt (in the discussion) about the origin of TCA.

8. Conclusions

A method for radiocarbon source identification of environmental chemicals present in soil at ppb levels using TCA as an exemplary compound was developed. The approach presented appears to be very powerful for differentiation between biogenic and anthropogenic sources of environmental chemicals. The chosen analyte was isolated in sufficient quantity in pure form, by a combination of differential liquid extraction steps, followed by derivatization, and two-dimensional PC-GC. In the first dimension a wide-bore high-capacity thick film capillary coated with a non-polar polysiloxane was used. For further removal of by-products the fraction containing the analyte was cryogenically collected, and the final purity refinement was effected on a short thin-film, high-efficiency capillary coated with a medium-polar polysiloxane. From a sequence of runs, the required amount of analyte was collected with a cryogenic fraction collector and combusted to CO_2 which was fed into the gas ionization source of an AMS-instrument.

Such an approach had already been applied for radiocarbon determination of individual environmental chemicals like fatty acids, n-alkanes, polycyclic aromatic hydrocarbons, or halogenated organic compounds after preparative isolation. The amount of 1.5 kg of estuarine sediment sample was enough for isolating 140 - 1190 μg of carbon in target compounds (Uchida et al., 2000). Fatty acids and n-alkanes were isolated from plant or petroleum samples in about 50 repeated chromatographic separations yielding 52 - 385 μg of carbon in individual compounds (Eglinton et al., 1996). In order to isolate 6 selected polycyclic aromatic hydrocarbons from atmospheric particulate material, over 100 injections had to be made yielding 35 - 251 μg of carbon (Currie et al., 1997). For the isolation of 150 μg of carbon obtained from halogenated bipyrroles originating from marine mammal and bird tissues over 100 injections were necessary (Reddy et al., 2004).

AMS is the only method which enables the direct determination of ^{14}C. The amount of carbon required depends on how the analyte is introduced into the ionization source and the overall ionization efficiency. With a conventional Cs-sputter source, 30 μg of elemental carbon are needed, with a titanium-target gas source 1 μg carbon in the form of carbon dioxide is sufficient. At such low levels, significant complicating factors are the ubiquitous chemical trace pollutants in laboratory air and the presence of atmospheric carbon dioxide containing natural occurring ^{14}C. Therefore,

great care must be taken to avoid blank contamination during sample preparation steps. As $^{14}CO_2$ and industrial organic trace pollutants are always present in laboratory air, on-line coupling of two-dimensional gas chromatographic, cryo-fractionation, combustion, and AMS-analyses is the ultimate goal to widen the applicability of the method for practical environmental problem solving.

For the determination of ^{14}C obtained from TCA in soil, the total amount of soil sample depends on its concentration in this compartment. As soil matrix is very inhomogenic, finding one sampling site with a high TCA burden does not assure the same TCA concentration in the whole sampling area a fact which must be taken into account as it can change the amount of sample required for analysis considerably. As TCA represents only a small fraction in the ppb range, great care must be taken to extract this analyte selectively from the very complex soil matrix. Such low concentrations of analyte enhance its swamping by co-eluting compounds present at much higher levels thus rendering separation of the analyte in one chromatographic run impossible.

To identify compounds co-eluting with TCA-OMe during preparative chromatographic separation, the Kovats Index of TCA-OMe was determined to be 922. The literature value determined at the same temperature on a home-made glass capillary (15 m x 0.22 mm i.d.) coated with methyl silicone, was 895.5 (Komarek et al., 1982). The values differ by 3 % which is mainly due to the differences in dimensions and stationary phase polarity of analytical columns used reflecting that retention times increase with increasing polarity of the stationary phase (Haken et al., 1981). As it was not possible to define the compound unequivocally by the Kovats index GC/MS was used to solve the problem. On the basis of the mass spectrum the co-eluting compound was identified to be hexanoic acid methyl ester. Its Kovats retention index determined at 150 °C on an aluminium column (3.66 m x 6.35 mm o.d.) packed with methyl silicone was 902 (Ashes et al., 1974). Knowing at least one of the interfering compounds helps to improve the extraction of TCA-OMe from soil samples.

The result obtained from AMS analysis, 2.2 PMC for TCA-OMe corresponding to 3.3 PMC for TCA, indicates without doubt that TCA stemming from soil in Oberthulba contains no ^{14}C and that it was formed from its anthropogenic precursors in the atmosphere and then was deposited in soil. This fact is of great importance for risk assessment procedures performed for TCA and tetrachloroethylene under the OECD and EU existing chemical programs. To reduce the influence of environmental

chemicals on several environmental compartments it must be known if they originate from anthropogenic sources. Only then it is possible to control emissions of their precursors and reduce their intrusion into the environment.

9. Summary

A method for radiocarbon source identification of environmental chemicals present in soil at ppb levels using trichloroacetic acid (TCA) as an exemplary compound was developed in the present thesis. A powerful approach to differentiate between biogenic and anthropogenic sources of environmental chemicals is to determine the radiocarbon content: an organic chemical derived from the biological carbon pool, and thus resulting from assimilation of atmospheric carbon dioxide, contains the radioactive carbon isotope ^{14}C at the typical atmospheric equilibrium abundance of $^{14}C/^{12}C = 1.2 \times 10^{-12}$, while a chemical derived from fossil carbon over 200 million years old is free of ^{14}C (half life 5730 years). The only method being sensitive enough for direct quantitative determination of a few thousand atoms of radiocarbon in a minute amount of a pure environmental trace chemical is accelerator mass spectrometry (AMS). In the present thesis an AMS equipped with a titanium-target gas source was used. Therefore, 1 μg carbon in the form of carbon dioxide was sufficient for the measurement.

To determine TCA in soil the total amount of soil sample needed depends on its concentration in this compartment. For sampling, sites of the Bavarian spruce site observational network were selected where TCA concentrations were relatively high. Two sampling sites were compared and the one with the higher TCA soil burden was chosen. Soil samples from Oberthulba were collected, and TCA was isolated in sufficient quantity in pure form, by a combination of differential liquid extraction steps, followed by derivatization, and preparative capillary-gas chromatography (PC-GC). In environmental samples the actual analyte constitutes usually only a minute fraction, mostly less than 0.1 % of the primary extract. Taking this into consideration, sequential (two-dimensional) PC-GC-separation on two different columns was necessary emphasizing high chromatographic capacity and high stationary phase selectivity in the first step and high separation efficiency in the second one. This was achieved by using two columns of appropriate dimensions. In the first dimension a wide-bore high-capacity thick film capillary coated with a non-polar stationary phase was used (coated with 1.5 μm d_f 95%-methyl-5%-phenyl-polysiloxane, CP Sil 8 CB, 60 m x 0.53 mm i.d.). For further removal of by-products the fraction containing the analyte was cryogenically collected, and the final purity refinement was effected on a short thin-film, high-efficiency capillary coated with a medium-polar stationary phase

(coated with 1.0 μm d_f 50 %-methyl-50 %-phenyl-polysiloxane, CP-Sil 24 CB, 30 m x 0.53 mm i.d.). From a sequence of runs, the required amount of trichloroacetic acid methyl ester (TCA-OMe) was collected with a cryogenic fraction collector, combusted to carbon dioxide, water and chlorine were separated, and carbon dioxide was fed into the gas ionization source of the AMS-instrument.

Great care was taken to avoid blank contamination during sample preparation steps for AMS because at such low levels significant complicating factors are the ubiquitous chemical trace pollutants in laboratory air and the presence of atmospheric carbon dioxide containing natural occurring ^{14}C.

The radiocarbon determination of a real sample of TCA collected in the mineral horizon of forest soil in Northern Bavaria yielded a value of 2.2 percent modern carbon (PMC) for TCA-OMe corresponding to 3.3 PMC for TCA indicating the anthropogenic origin of TCA. If TCA were of biogenic origin, a value of 66.7 PMC would be expected as one additional carbon from a petrochemical source is introduced during derivatization to the methyl ester. The result proved that the TCA found in the soil at the particular sampling location was an atmospheric breakdown product of tetrachloroethylene and not a product of any purported soil microbial process. This result is important for risk assessment procedures performed for TCA and tetrachloroethylene under OECD and EU chemical programs. To control emissions of environmental chemicals and to reduce their influence on the various environmental compartments it must be known if they originate from anthropogenic sources.

10. Zusammenfassung

In der vorliegenden Dissertation wurde die Radiocarbonmethode zur Quellenidentifizierung von Umweltchemikalien, die im Boden in ppb Konzentrationen auftreten, angewandt. Als repräsentative Umweltchemikalie diente Trichloressigsäure (TCA). Eine leistungsfähige Methode, um zwischen biogenen und anthropogenen Quellen von Umweltchemikalien zu differenzieren, ist die Radiokarbongehalt-Bestimmung: eine organische Verbindung, die biogenen Ursprungs und somit ein Produkt der atmosphärischen Kohlendioxid-Assimilation ist, enthält das radioaktive Kohlenstoff-Isotop ^{14}C in der für die Atmosphäre typischen Menge ($^{14}C/^{12}C = 1{,}2 \times 10^{-12}$), dagegen ist eine Verbindung, die aus fossilem Kohlenstoff stammt, der 200 Millionen Jahre alt ist, ^{14}C-frei (Halbwertzeit 5730 Jahre). Die einzige Methode, die zur direkten quantitativen Bestimmung einiger tausend ^{14}C-Atome in einer reinen Umweltchemikalie, die nur in kleiner Menge vorliegt, empfindlich genug ist, ist die Beschleuniger-Massenspektrometrie (AMS). In der vorliegenden Dissertation wurde ein AMS-Instrument genutzt, das mit einer Titan-Target Gasionenquelle ausgerüstet ist. Deswegen war 1 μg Kohlenstoff in Form von Kohlendioxid für die Messung ausreichend.

Bei der Bestimmung von TCA in Bodenproben hängt die Probenmenge von der TCA Konzentration in diesem Kompartiment ab. Aus dem Fichten-Monitoringnetz des Landesamtes für Umweltschutz in Bayern wurden Probenahmeorte ausgewählt, an denen die höchsten TCA-Konzentrationen in Fichtennadeln gefunden worden waren. Bodenproben wurden in Oberthulba genommen, und TCA wurde in ausreichender Menge und reiner Form über mehrere wässrige Extraktionsstufen, Derivatisierung und präparative Kapillar-Gaschromatographie (PC-GC) isoliert. Die sequentielle zwei-dimensionale PC-GC-Trennung auf zwei verschiedenen Säulen war nötig, weil der Analyt in Umweltproben nur eine kleine Fraktion, meistens weniger als 0,1 % des primären Extraktes, ausmacht. Bei der Trennung waren sowohl die hohe chromatographische Kapazität und Selektivität der stationären Phase im ersten Schritt als auch die hohe Trennungseffizienz im zweiten Schritt wichtig. Dies wurde mit zwei Säulen mit geeigneten Dimensionen erreicht. In der ersten Dimension wurde eine Kapillare mit hoher Kapazität und großem Durchmesser benutzt, die mit einem dicken Film der unpolaren stationären Phase beschichtet ist (1,5 μm d_f 95%-Methyl-5%-phenyl-polysiloxan, CP Sil 8 CB, 60 m x 0,53 mm i.D.). Zur Entfernung der Ne-

benprodukte wurde die den Analyten enthaltende Fraktion unter Kühlung (-80 °C) gesammelt, die endgültige Reinigung wurde auf einer kurzen Kapillare durchgeführt, die mit einem dünnen Film der mittelpolaren stationären Phase beschichtet ist (1,0 µm d_f 50 %-Methyl-50 %-phenyl-polysiloxan, CP-Sil 24 CB, 30 m x 0,53 mm i.D.). Es wurden mehrere Injektionen durchgeführt, um die für die AMS-Bestimmung benötigte Menge Trichloressigsäure-Methylester (TCA-OMe) in reiner Form zu erhalten. TCA-OMe wurde bei -80 °C in einem Fraktionssammler gesammelt und anschließend zu Kohlendioxid verbrannt. Bei der Verbrennung entstehen neben Kohlendioxid auch Wasser und Chlor, die in einem Hochvakuum-System entfernt wurden. Danach wurde das Kohlendioxid in die Gasionenquelle des AMS-Instruments geleitet.

Sorgfältig wurde die Kontamination während der Probenaufarbeitung für die AMS-Bestimmung vermieden, weil sowohl ubiquitär in der Laborluft auftretende chemische Spurenfremdstoffe als auch atmosphärisches Kohlendioxid, das ^{14}C enthält, Verunreinigungsquellen darstellen, die beim Arbeiten in so niedrigen Konzentrationsbereichen die Ergebnisse verfälschen können.

Die ^{14}C-Bestimmung von TCA in der Realprobe, die im mineralischen Horizont des Waldbodens in Nordbayern genommen worden war, ergab einen Wert von 2,2 Prozent moderner Kohlenstoff (PMC) für TCA-OMe, dies entspricht einem Wert von 3,3 PMC für TCA. Dieser PMC-Wert zeigt deutlich, dass TCA anthropogenen Ursprungs ist. Wäre TCA biogenen Ursprungs, sollte der Wert 66,7 PMC betragen, da durch die Derivatisierung zum Methylester ein zusätzliches petrochemisches Kohlenstoffatom eingeführt wurde. Das Ergebnis zeigt eindeutig, dass TCA, die im Boden des Probenahmeortes gefunden wurde, ein atmosphärisches Degradationsprodukt des Tetrachlorethens ist und nicht - wie postuliert - das Produkt deines im Boden ablaufenden mikrobiellen Prozesses. Dieses Ergebnis ist von großer Bedeutung für die Risikoabschätzungen von TCA und Tetrachlorethen, die von der OECD und EU vorgenommen werden. Es ist wichtig zu wissen, ob Umweltchemikalien anthropogenen oder biogenen Ursprungs sind, um ihre Emissionen und ihren Einfluss auf verschiedene Umweltkompartimente kontrollieren zu können.

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Appendix 1: Raw data from AMS measurement

Run	Target name	C14 [counting rates]	C13 [nA]	C12 [nA]	V14/13	V13/12
1	Graphite + Fe	4	111.59	10530.94	0.03585	0.0106
2	Graphite + Fe	4	117.37	11092.57	0.03408	0.01058
3	Graphite + Fe	4	120.63	11407.71	0.03316	0.01057
4	Graphite + Fe	7	122.2	11575.45	0.05728	0.01056
5	Graphite + Fe	5	123.05	11667.27	0.04063	0.01055
6	Graphite + Fe	6	118.31	11185.16	0.05071	0.01058
7	Graphite + Fe	6	125.09	11829.69	0.04797	0.01057
8	Graphite + Fe	8	125.14	11833.52	0.06393	0.01058
9	Graphite + Fe	7	122.99	11586.5	0.05692	0.01061
10	Graphite + Fe	11	125.62	11824.4	0.08757	0.01062
11	Graphite + Fe	5	127.86	12040.84	0.03911	0.01062
12	Graphite + Fe	6	128.68	12106.71	0.04663	0.01063
13	Graphite + Fe	7	127.84	12022.69	0.05476	0.01063
14	Graphite + Fe	3	127.72	12043.26	0.02349	0.01061
15	Graphite + Fe	2	130.72	12336.59	0.0153	0.0106
17	Ox II	3690	159	14808.81	23.2075	0.01074
18	Ox II	3547	158	14729.03	22.4494	0.01073
19	Ox II	3679	158.73	14813.08	23.1777	0.01072
20	Ox II	3555	159.44	14874.47	22.2968	0.01072
21	Ox II	3681	160.68	15012.62	22.9089	0.0107
22	Ox II	3768	162.42	15172.98	23.1991	0.0107
23	Ox II	3810	164.24	15337.88	23.1978	0.01071
24	Ox II	3869	165.4	15465.38	23.3918	0.01069
25	Ox II	3822	166.41	15575.39	22.9674	0.01068
26	Ox II	3740	167.38	15681.98	22.3444	0.01067
27	Ox II	3829	167.96	15761.46	22.7971	0.01066
28	Ox II	3786	168.49	15813.18	22.4702	0.01066
29	Ox II	3818	168.97	15886.17	22.5957	0.01064
30	Ox II	3941	170.23	15999.91	23.151	0.01064
31	Ox II	3965	170.65	16038.11	23.2347	0.01064
33	Ox 50%	1292	149.67	14095.11	8.63232	0.01062
34	Ox 50%	1336	149.81	14134.75	8.91796	0.0106

Run	Target name	C14 [counting rates]	C13 [nA]	C12 [nA]	V14/13	V13/12
35	Ox 50%	1256	150.88	14238.01	8.3245	0.0106
36	Ox 50%	1303	152.49	14416.2	8.54482	0.01058
37	Ox 50%	1325	154.32	14602.5	8.58605	0.01057
38	Ox 50%	1303	156.18	14782.7	8.34294	0.01057
39	Ox 50%	1378	157.56	14937.06	8.74587	0.01055
40	Ox 50%	1329	158.44	15037.87	8.38803	0.01054
41	Ox 50%	1384	160.06	15203.62	8.64676	0.01053
42	Ox 50%	1361	162.16	15415.13	8.39295	0.01052
43	Ox 50%	1399	164.29	15637.15	8.51543	0.01051
44	Ox 50%	1507	178.36	16588.85	8.4492	0.01075
45	Ox 50%	1595	183.66	17192.25	8.68453	0.01068
46	Ox 50%	1583	181.34	16990.91	8.72946	0.01067
47	Ox 50%	1489	178.34	16753.1	8.34922	0.01065
49	IAEA-C5	616	156.62	14639.31	3.93309	0.0107
50	IAEA-C5	622	154.91	14474.74	4.01523	0.0107
51	IAEA-C5	597	156.23	14695.91	3.82129	0.01063
52	IAEA-C5	569	157.7	14892.18	3.60812	0.01059
53	IAEA-C5	603	157.78	14868.82	3.82178	0.01061
54	IAEA-C5	585	158.45	14900.65	3.69202	0.01063
55	IAEA-C5	655	160.69	15211.33	4.07617	0.01056
56	IAEA-C5	658	162.27	15411.28	4.05497	0.01053
57	IAEA-C5	635	162.96	15448.74	3.89666	0.01055
58	IAEA-C5	671	163.48	15482.85	4.10448	0.01056
59	IAEA-C5	637	165.25	15715.24	3.85477	0.01052
60	IAEA-C5	641	166.11	15814.44	3.85889	0.0105
61	IAEA-C5	668	169.28	16189.21	3.94612	0.01046
62	IAEA-C5	677	175.69	16926.1	3.85338	0.01038
63	IAEA-C5	685	180.96	17453.15	3.78537	0.01037
100	Graphite	1	1.93	131.24	0.51813	0.01471
101	Graphite	2	5.04	377.5585	0.39683	0.01335
102	Graphite	4	12.17	909.9597	0.32868	0.01337
103	Graphite	2	14.38	1002.467	0.13908	0.01434

Run	Target name	C14 [counting rates]	C13 [nA]	C12 [nA]	V14/13	V13/12
104	Graphite	10	17.69	1273.58	0.56529	0.01389
105	Graphite	11	33.13	2607.201	0.33203	0.01271
106	Graphite	17	44.43	3587.54	0.38262	0.01238
107	Graphite	8	45.29	3636.781	0.17664	0.01245
108	Graphite	19	45.08	3617.874	0.42147	0.01246
109	Graphite	11	41.93	3355.449	0.26234	0.0125
110	Graphite	8	33.51	2623.838	0.23873	0.01277
111	Graphite	7	31.03	2422.836	0.22559	0.01281
112	Graphite	15	37.03	2927.902	0.40508	0.01265
113	Graphite	6	39.15	3119.971	0.15326	0.01255
114	Graphite	14	35.35	2789.206	0.39604	0.01267
115	Graphite	10	24.53	1877.095	0.40766	0.01307
116	Graphite	8	16.17	1179.296	0.49474	0.01371
117	Graphite	2	12.84	921.6533	0.15576	0.01393
118	Graphite	0	11.28	804.8972	0	0.01401
119	Graphite	4	10.08	692.7979	0.39683	0.01455
120	Graphite	2	9.19	627.8191	0.21763	0.01464
121	Graphite	0	8.32	568.1417	0	0.01464
122	Graphite	3	7.54	509.2311	0.39788	0.01481
123	Graphite	0	6.96	464.7628	0	0.01498
124	Graphite	3	6.42	424.9853	0.46729	0.01511
125	Graphite	2	5.85	367.6659	0.34188	0.01591
126	Graphite	4	5.28	317.5636	0.75758	0.01663
127	Graphite	0	4.82	269.8899	0	0.01786
128	Graphite	2	4.48	244.6171	0.44643	0.01831
129	Graphite	1	4.2	227.337	0.2381	0.01847
130	Graphite	1	3.99	215.9958	0.25063	0.01847
131	Graphite	0	3.77	198.9201	0	0.01895
132	Graphite	2	3.64	188.2265	0.54945	0.01934
133	Graphite	0	3.43	180.4895	0	0.019
134	Graphite	0	3.22	168.2197	0	0.01914
135	Graphite	0	3.11	153.9977	0	0.0202

Run	Target name	C14 [counting rates]	C13 [nA]	C12 [nA]	V14/13	V13/12
202	Ox 50%	9	1.08	66.51401	8.33333	0.01624
203	Ox 50%	127	32.64	3027.271	3.89093	0.01078
204	Ox 50%	215	46.86	4385.017	4.58813	0.01069
205	Ox 50%	173	47.77	4461.992	3.62152	0.01071
206	Ox 50%	198	49.09	4600.574	4.03341	0.01067
207	Ox 50%	207	48.06	4514.776	4.30712	0.01065
208	Ox 50%	213	49.23	4616.608	4.32663	0.01066
209	Ox 50%	218	48.37	4535.068	4.50693	0.01067
210	Ox 50%	193	44.91	4200.695	4.29748	0.01069
211	Ox 50%	164	42.73	3997.171	3.83805	0.01069
212	Ox 50%	156	41.61	3889.014	3.7491	0.0107
213	Ox 50%	160	38.22	3567.312	4.18629	0.01071
214	Ox 50%	129	31.93	2952.368	4.04009	0.01082
215	Ox 50%	122	27.04	2468.544	4.51183	0.01095
216	Ox 50%	96	23.59	2119.969	4.06952	0.01113
217	Ox 50%	84	20.76	1848.142	4.04624	0.01123
218	Ox 50%	82	18.05	1593.506	4.54294	0.01133
219	Ox 50%	60	16.13	1400.149	3.71978	0.01152
220	Ox 50%	61	14.62	1254.414	4.17237	0.01165
221	Ox 50%	55	13.44	1133.572	4.09226	0.01186
222	Ox 50%	51	12.4	1042.694	4.1129	0.01189
257	Umwelt 09/04-2	10	45.33	4299.675	0.2206	0.01054
258	Umwelt 09/04-2	7	46.68	4420.788	0.14996	0.01056
259	Umwelt 09/04-2	14	44.53	4207.228	0.31439	0.01058
260	Umwelt 09/04-2	17	46.99	4511.427	0.36178	0.01042
261	Umwelt 09/04-2	2	26.05	2469.163	0.07678	0.01055
262	Umwelt 09/04-2	1	8.21	728.744	0.1218	0.01127
263	Umwelt 09/04-2	0	6.16	542.6406	0	0.01135
264	Umwelt 09/04-2	1	8.55	757.4111	0.11696	0.01129
265	Umwelt 09/04-2	5	17.59	1629.924	0.28425	0.01079
266	Umwelt 09/04-2	7	31.82	3022.999	0.21999	0.01053
267	Umwelt 09/04-2	12	39.26	3735.403	0.30565	0.01051

Run	Target name	C14 [counting rates]	C13 [nA]	C12 [nA]	V14/13	V13/12
268	Umwelt 09/04-2	6	30.84	2873.732	0.19455	0.01073
269	Umwelt 09/04-2	3	20.72	1877.522	0.14479	0.01104
270	Umwelt 09/04-2	7	18.78	1684.145	0.37274	0.01115
271	Umwelt 09/04-2	2	16.1	1438.026	0.12422	0.0112
272	Umwelt 09/04-2	1	15.34	1354.965	0.06519	0.01132
273	Umwelt 09/04-2	4	13.78	1209.273	0.29028	0.0114
322	Umwelt 08/04-3	0	2.72	224.9213	0	0.01209
323	Umwelt 08/04-3	5	12.68	1123.125	0.39432	0.01129
324	Umwelt 08/04-3	5	18.68	1714.322	0.26767	0.0109
325	Umwelt 08/04-3	4	20.5	1887.686	0.19512	0.01086
326	Umwelt 08/04-3	2	19.19	1767.203	0.10422	0.01086
327	Umwelt 08/04-3	3	16.02	1448.62	0.18727	0.01106
328	Umwelt 08/04-3	1	12.51	1105.888	0.07994	0.01131
329	Umwelt 08/04-3	4	9.71	840.4585	0.41195	0.01155
330	Umwelt 08/04-3	1	7.61	647.5944	0.13141	0.01175
331	Umwelt 08/04-3	2	5.97	503.3575	0.33501	0.01186
332	Umwelt 08/04-3	2	4.93	409.2069	0.40568	0.01205
333	Umwelt 08/04-3	0	4.36	360.057	0	0.01211
334	Umwelt 08/04-3	2	4.05	335.1421	0.49383	0.01208
335	Umwelt 08/04-3	0	3.94	322.5142	0	0.01222
336	Umwelt 08/04-3	0	3.87	318.8078	0	0.01214
337	Umwelt 08/04-3	0	3.82	312.664	0	0.01222
338	Umwelt 08/04-3	0	3.75	308.0969	0	0.01217
339	Umwelt 08/04-3	1	3.66	302.1837	0.27322	0.01211
488	Umwelt 09/04-4	0	0.8	46.96051	0	0.01704
489	Umwelt 09/04-4	1	2.83	228.7003	0.35336	0.01237
490	Umwelt 09/04-4	1	3.19	262.7409	0.31348	0.01214
491	Umwelt 09/04-4	2	5.23	437.103	0.38241	0.01197
492	Umwelt 09/04-4	3	9.31	806.7903	0.32223	0.01154
493	Umwelt 09/04-4	6	6.55	547.9921	0.91603	0.01195
494	Umwelt 09/04-4	1	3.72	299.0905	0.26882	0.01244
495	Umwelt 09/04-4	0	2.72	210.8492	0	0.0129

Run	Target name	C14 [counting rates]	C13 [nA]	C12 [nA]	V14/13	V13/12
496	Umwelt 09/04-4	0	2.28	169.8385	0	0.01342
497	Umwelt 09/04-4	1	2.05	145.7418	0.4878	0.01407
498	Umwelt 09/04-4	0	2.08	153.4787	0	0.01355
499	Umwelt 09/04-4	1	5.16	429.7349	0.1938	0.01201
500	Umwelt 09/04-4	15	28.31	2683.556	0.52985	0.01055
501	Umwelt 09/04-4	4	10.35	940.6813	0.38647	0.011
502	Umwelt 09/04-4	5	10.22	931.8667	0.48924	0.01097
503	Umwelt 09/04-4	9	19.64	1832.915	0.45825	0.01072
504	Umwelt 09/04-4	18	32.84	3114.474	0.54811	0.01054
505	Umwelt 09/04-4	16	27.05	2527.199	0.5915	0.0107
506	Umwelt 09/04-4	8	12.76	1139.469	0.62696	0.0112
507	Umwelt 09/04-4	4	10.2	891.3932	0.39216	0.01144
508	Umwelt 09/04-4	5	8.77	757.2877	0.57013	0.01158
509	Umwelt 09/04-4	1	7.86	668.7125	0.12723	0.01175
510	Umwelt 09/04-4	2	7.13	599.9818	0.2805	0.01188
511	Umwelt 09/04-4	2	6.55	549.9741	0.30534	0.01191
512	Umwelt 09/04-4	5	5.88	486.5809	0.85034	0.01208
513	Umwelt 09/04-4	3	5.35	438.6337	0.56075	0.0122
514	Umwelt 09/04-4	2	4.95	401.6671	0.40404	0.01232
515	Umwelt 09/04-4	5	4.67	374.4711	1.07066	0.01247
516	Umwelt 09/04-4	2	4.38	350.0612	0.45662	0.01251
517	Umwelt 09/04-4	6	4.16	329.0636	1.44231	0.01264
518	Umwelt 09/04-4	2	3.99	310.8426	0.50125	0.01284
519	Umwelt 09/04-4	3	3.87	301.4405	0.77519	0.01284
520	Umwelt 09/04-4	2	3.79	293.3052	0.5277	0.01292
550	Ox II	1634	141.17	13570.14	11.5747	0.0104
551	Ox II	1652	144.3	13792.04	11.4484	0.01046
552	Ox II	1707	146.92	14116.49	11.6186	0.01041
553	Ox II	1742	150.96	14428.81	11.5395	0.01046
554	Ox II	3474	154.89	14815.81	22.4288	0.01045
555	Ox II	3655	159.21	15221.7	22.9571	0.01046
556	Ox II	3792	163.4	15620.98	23.2069	0.01046

Run	Target name	C14 [counting rates]	C13 [nA]	C12 [nA]	V14/13	V13/12
557	Ox II	3772	161.02	15352.13	23.4257	0.01049
558	Ox II	3161	136.84	12977.74	23.1	0.01054

Erklärung:

Hiermit erkläre ich, dass ich die Arbeit selbständig verfasst und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe.
Ferner erkläre ich, dass ich anderweitig mit oder ohne Erfolg nicht versucht habe, diese Dissertation einzureichen oder mich der Doktorprüfung zu unterziehen.

Bayreuth, den 27. Februar 2006