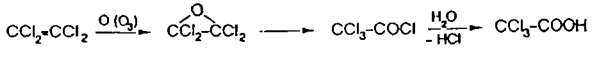
# DETERMINATION OF TCA IN SPRUCE NEEDLES

H. Frank, H. Scholl
Institut für Toxikologie
Universität Tübingen
7400 Tübingen, Federal Republic of Germany

ABSTRACT Trichloroacetic acid (TCA) has been determined quantitatively in spruce needles from the Black Forest by capillary gas chromatography. The concentrations of TCA vary between 0 ppb to 140 ppb, depending upon season, location and age of the needles.

## INTRODUCTION

Trichloroacetic acid (TCA) is known as a strong herbicide. $C_2$-chlorocarbons such as tetrachloroethene and 1,1,1-trichloroethane are degraded by atmospheric oxidation to TCA (fig.1).



TCA occurs in soil and foliage of trees in relatively low-polluted mountain ranges. The formation of the ubiquitous herbicide from airborne $C_2$-chlorocarbons poses an environmental hazard and is possibly one of the causes of forest decline. Although the load of forest trees is lower than typical for direct herbicide application to weeds, the phenomena of chronic phytotoxicity elicited by continuous low-level exposure have not been studied so far. In order to establish proper dose-regimens for phytotoxicological experiments, typical TCA-levels at representative forest sites must be known.

Various methods for TCA-analysis have been published. The oldest one (Fujiwara 1916) is based upon the formation of a coloured reaction product from chloroform and pyridine in alkaline medium. Chloroform arises from decarboxylation of TCA under heating; detection limit of this method is about 100 mg/kg. Newer methods are mostly based on gas chromatography. TCA can be decarboxylated to chloroform, which is determinated by headspace gas chromatography/ECD; the advantage of this method is speed. However, no internal standard is used, and chloroform present in ambient air may interfere.

Extraction of TCA and derivatization with diazomethane to its methylester is more reliable, since an internal standard is employed; on the other hand, sample work-up is considerably more time-consuming.

## EXPERIMENTAL PART

Sample collection: The needles are cut off the twigs with a pair of scissors; age groups are kept separate. They are collected in screw-cap vials and transported on ice to the laboratory, where they are kept at -40°C until further preparation.

Sample preparation: Needles are pulverized under liquid nitrogen. TCA is extracted from aliquots of the homogeneous matrix with deionized water. Dichloropropionic acid (DCP) is added as internal standard.

The samples are acidified with sulphuric acid to pH 0 and re-extracted with ether. For methylation of TCA and the internal standard, the ether phase is mixed with an ethereal saturated diazomethane-solution .

Gas Chromatography: A sample aliquot of 1.0 µl of the derivatized ether extract is injected into a gas chromatograph (Carlo Erba, mod. 4160) equipped with a split-injector, a silanized glass capillary (23 m * 0.3 mm) coated with 2.0 µm immobilized methyl-phenyl(5%)-vinyl(1%)-silicone (SE 54), and an electron capture detector (Carlo Erba HT25).

Carrier gas is purified hydrogen at an inlet pressure of 28 kPa. Initial oven temperature is 40°C. One minute after splitless injection, the split valve is opened to a ratio of 1:10; the oven temperature is raised to 150°C at a rate of 10°C/min, to 210°C at a rate of 30°C/min and held for 1 minute. Injector temperature is 180°C, detector temperature is 220°C. Scavenger gas is argon/methane (9/1) at a flow rate of 26 ml/min, working temperature of the ECD is 275°C, mode is constant current with a pulse width of 0.1 µs. Quantitative determination is based upon integration of the TCA- and DCP-peaks with an integration program (type 2600, PE Nelson, Sinsheim, FRG).

For calibration, a standard of sodium trichloroacetate is diluted with water to concentrations of 4, 8, 12, 16, 20 and 40 ng TCA/ml. Aliquots of 4 ml are prepared in the same way as the water extracts of needles.

## RESULTS AND DISCUSSION

TCA-levels in needles from trees of mountain forest sites in Southwest Germany are shown in table 1.

The concentrations of TCA varies from 0 µg/kg to 140 µg/kg, with most measurements between 20 µg/kg and 40 µg/kg. There is an increase during the summer months. Highest levels are reached in autumn; they decrease in winter. Older needles are usually higher loaded than younger one.

Whether the decrease of TCA in the plants is due to metabolism or mobilization is so far unknown. Trichloroacetic acid may be taken up from soil via the roots or via the leaves from the atmosphere. To which extent TCA is formed

from tetrachloroethene by plant metabolism remains to be elucidated. Exposure experiments have been started to study the phytotoxic effect of airborne halogenated hydrocarbons and to determine their role in forest decline by establishing dose-response relations.

TABLE 1: TCA - levels in needles of MaTa: Mauzenberg, fir; MaFi: Mauzenberg, spruce; BeFi: Bernstein, spruce; ScFi: Schwarzwald Hochstraße, spruce; ZwTa: Zwieselberg, fir; ZwFi: Zwieselberg, spruce; HeFi: Herrenberg, spruce; SöFi: Schönbuch, spruce

| needle age | MaTa 87/89 | MaFi 87/89 | BeFi 87/89 | ScFi 87/89 | ZwTa 87/89 | ZwFi 87/89 | HeFi 87/89 | SöFi 87/89 |
|---|---|---|---|---|---|---|---|---|
| 05.05.89 | | 24/8 | 31/4 | 11/3 | | | | 14/5 |
| 26.06.89 | 10/5 | 12/7 | 18/10 | 12/7 | 7/- | | | 13/3 |
| 17.07.89 | 13/2 | 14/4 | 42/12 | 13/11 | 18/16 | | | 18/0 |
| 08.08.89 | 23/19 | 22/18 | 75/29 | 17/14 | 15/17 | | | 18/7 |
| 30.08.89 | 21/10 | 11/8 | 75/59 | 13/22 | 14/11 | 20/7 | | 19/7 |
| 28.09.89 | 22/11 | 44/17 | 83/68 | 17/23 | 12/16 | 19/17 | | 22/16 |
| 19.10.89 | 14/9 | 11/9 | 97/65 | 11/5 | 11/12 | 14/4 | | -/12 |
| 08.11.89 | 10/9 | 11/9 | 80/62 | 15/8 | 11/11 | 20/15 | | 15/- |
| 12.12.89 | 9/7 | 9/6 | 47/31 | 12/6 | 13/7 | 25/11 | | 12/12 |
| 02.01.90 | 19/19 | 32/39 | 23/24 | 32/27 | 20/28 | 28/21 | | 32/47 |
| 05.02.90 | 20/20 | 27/42 | 33/20 | 18/14 | 19/18 | 25/20 | 16/14 | 26/32 |
| 30.04.90 | 9/11 | 5/6 | 8/10 | 5/6 | 6/6 | 9/14 | 7/9 | 12/15 |
| needle age | 88/90 | 88/90 | 88/90 | 88/90 | 88/90 | 88/90 | 88/90 | 88/90 |
| 06.06.90 | 9/7 | 15/13 | 16/7 | 7/5 | 9/6 | 7/3 | 10/2 | 14/3 |
| 11.07.90 | 23/10 | 20/9 | 31/11 | 14/6 | 12/16 | 9/7 | 20/11 | 11/11 |

## REFERENCES


Frank, H.; Frank, W. Environ. Sci. Technol 1989, 23, 365

Frank, H.; Nachr. Chem. Tech. Lab. 1988, 36, 889

Frank, H.; Frank, W.; Thiel, D. Atmos. Environ. 1989, 23, 1333

Ashton, F.M.; Crafts, A.S. " Mode of Action of Herbicides", Wiley Interscience 1973

Frank, H.; Frank, W. Naturwissenschaften 1985, 11, 139

Müller, G. et al. Arch. Toxicol. 1972, 29, 335

Reiss, J. Diplomarbeit Uni Tübingen 1990

Fujiwara, K. Sitz. Nat. Ges. Rostock 1916, 6, 33