Groundwater contaminations – the use of LC-NMR and LC-MS to characterize the scope of polar contaminants

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Abstract

Organic pollutants that are released into the environment are subjected to various chemical, photochemical and microbiological transformation processes. As a result, a variety of new and unexpected transformation products can be formed and, as a rule, they are more polar than the parent compounds. While the parent compounds and some of their known metabolites are analyzed with optimized analytical methods (target analysis) unknown transformation products and metabolites have often been overlooked in the past. Today, the combined use of LC-NMR and LC-MS techniques offer the possibility to identify unknown compounds in environmental samples routinely (non-target analysis). General aspects of this new analytical approach are discussed and examples of application are reported, which cover the characterization of polar explosives and related compounds in groundwater samples from ammunition waste sites. Via the example of biodegradation of mononitrotoluenes, it is further shown how non-target analysis can complement the investigation of the fate of environmental pollutants.
Keywords: non-target analysis, LC-NMR, LC-MS, explosives and related compounds.

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1 Introduction

In the past, the first substances analyzed were the parent pollutants that were originally released into the environment. Later on, in some cases, important known transformation products were included in the characterization of environmental samples. Since the chemical and physical properties of these compounds were known in most cases, specific analytical methods for their determination in air, water, and soil could be developed (target analysis).

For transformation products and metabolites, however, the situation is different. Generally, these compounds were not analyzed, because in most cases they are not regulated and no effective analytical methods exist for their determination. This means that a correct diagnosis of the environmental situation cannot be made and, as a consequence, no appropriate action can be taken. Therefore, in order to improve the risk assessment of hazardous waste sites and to get insights into the environmental fate of pollutants the scope of pollutants should be analyzed as comprehensively as possible. For this task methods should be used which combine high separation efficiency with a maximum of structural information. These are hyphenated techniques such as GC-MS, LC-MS, and LC-NMR. It is the intention of this presentation to demonstrate the possibilities that the combined use of LC-NMR and LC-MS offers, sometimes completed by further off-line NMR and MS investigations, for a fast characterization of unknown polar pollutants and metabolites in environmental samples (non-target analysis). This is not only useful for the development of reliable analytical methods used in routine analysis, but can also provide information on the environmental fate of pollutants.

2 Methods

1-2 L of the aqueous sample adjusted to pH 7 were extracted three times with 40 ml dichloromethane to remove the less polar components. The aqueous phase was adjusted to pH 2 with phosphoric acid and sucked over a LiChrolut EN cartridge conditioned with 3 mL methanol, 3 mL acetonitrile and 10 mL water. The cartridge was left dripping wet and the analytes were eluted with 2 x 3 mL acetonitrile/water (80/20). Depending on the analyte concentration the eluate was further reduced to 1,0-0.05 mL for the on-flow LC-NMR runs.

LC-NMR investigations were performed using a Bruker DRX 600 NMR spectrometer equipped with a ^{1}H-^{13}C inverse LC probe head (4 mm i.d., detection volume 120 µl). The chromatographic system connected to the DRX 600 consisted of a Bruker LC 22 pump, a Bruker BPSU 36 peak sampling unit, and a Bischoff UV detector Lamda 1000. For the LC-MS investigations a Bruker LCQ equipped with an ESI ion source was used. For further experimental details refer to the original literature cited in the presented examples.

Table 1: Abbreviations used for polar compounds identified in groundwater samples from former ammunition production sites.

Abbreviation	Full name
Nitroaromatic compounds	
4-NP	4-nitrophenol
2,4-NP	2,4-dinitrophenol
3,5-DNP	3,5-dinitrophenol
3-M-2,6-DNP	3-methyl-2,6-dinitrophenol
4-M-2,6-DNP	4-methyl-2,6-dinitrophenol
5-M-2,4-DNP	5-methyl-2,4-dinitrophenol
3-M-2,4,6-TNP	3-methyl-2,4,6-trinitrophenol
2-NBAl	2-nitrobenzyl alcohol
4-NBAl	4-nitrobenzyl alcohol
BA	benzoic acid
4-M-BA	4-methylbenzoic acid
2-NBA	2-nitrobenzoic acid
3-NBA	3-nitrobenzoic acid
4-NBA	4-nitrobenzoic acid
4-ABA	4-aminobenzoic acid
2,4-DNBA	2,4-dinitrobenzoic acid
2-M-3-NBA	2-methyl-3-nitrobenzoic acid
3-M-2-NBA	3-methyl-2-nitrobenzoic acid
3-M-4-NBA	3-methyl-4-nitrobenzoic acid
4-M-2-NBA	4-methyl-2-nitrobenzoic acid
4-M-3-NBA	4-methyl-3-nitrobenzoic acid
5-OH-2-NBA	5-hydroxy-2-nitrobenzoic acid
5-OH-2,4-DNBA	5-hydroxy-2,4-dinitrobenzoic acid
2,4-DNT-3-SA	2,4-dinitrotoluene-3-sulfonic acid
2,4-DNT-5-SA	2,4-dinitrotoluene-5-sulfonic acid
Nitramines	
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
MNX	hexahydro-1,3-dinitro-5-nitroso-1,3,5-triazine
mn-HMX	octahydro-1,3,5-trinitro-7-nitroso-1,3,5,7-tetrazocine
SEX	octahydro-1N-acetyl-3,5,7-trinitro-1,3,5,7-tetrazocine
Derivatives of quinolinyl-3-acetic acid	
2-O-1,2,3,4-THQ-3-AA	2-oxo-1,2,3,4-tetrahydroquinolinyl-3-acetic acid
2-O-1,2-DHQ-3-AA	2-oxo-1,2-dihydroquinolinyl-3-acetic acid
2-O-4-OH-1,2-DHQ-3-AA	2-oxo-4-hydroxy-1,2-dihydroquinolinyl-3-acetic acid

3 Results and discussion

3.1 General aspects of non-target analysis

Non-target analysis means the identification and structure elucidation of unknown organic components in environmental samples. It is much more difficult to perform than target analysis. Sample extraction and separation must take into account the very different (and a priori unknown) physical and chemical properties of the individual organic compounds, while the detection methods must provide a maximum of structural information. Furthermore, the separation of complex mixtures is often incomplete, especially when liquid chromatography is used. For compounds amenable to GC (volatile and semi-volatile thermally stable compounds), the situation is less critical, since the separation efficiency of gas chromatography is high and the mass detector under electron impact conditions provides valuable structural information.

Polar compounds can better be separated by HPLC, but for a long time only UV or PDA detectors were available, and the structural information of a UV spectrum is completely insufficient for non-target analysis.

3.2 Non-target analysis by LC-MS

Over the past fifteen years, LC-MS coupling has been developed as a routine method for target analysis of polar compounds. Due to the extended possibilities offered by MS/MS and MS^n techniques, it has also been used increasingly for the identification of unknown compounds. Regarding non-target analysis, the LC-MS method has the following features:

Advantages:

1. The mass detector provides the important information on the quasi-molecular ions (e.g. $[M+H]^+$ and $[M-H]^-$).
2. Functional groups (e.g. halogens, COOH, NO_2, SO_3H) are readily detected either via the isotope pattern or characteristic neutral losses in MS/MS experiments.

Disadvantages:

1. The possibilities for structural elucidation of unknowns are restricted; in particular, a distinction between isomers is possible only in exceptional cases.
2. There are no universal ionization conditions under which any unknown compound can be expected to be ionized, and the ionization efficiency depends strongly on the chemical properties of the analytes.
3. For quantification of the identified compounds, the genuine reference compounds (isotope-labeled) are needed, which are often not available for transformation products and metabolites.

3.3 Non-target analysis by LC-NMR

Besides mass spectrometry, nuclear magnetic resonance (NMR) spectroscopy is the second important method for structural analysis and the information it yields is often complementary to that of mass spectrometry. It therefore seems very

meaningful to also use LC-NMR for non-target analysis of complex environmental samples. In the past, this failed because of the low sensitivity of the NMR spectrometer and the limited dynamic range of the digitizer and receiver, which did not allow very weak analyte signals to be detected in the presence of strong solvent signals. Today, the sensitivity of a high-field NMR spectrometer (> 500 MHz) is much higher, and cryoprobes, which have been commercially available for some years, have led to a further increase in sensitivity. There are also a number of very efficient methods for suppression of the solvent signals. Regarding non-target analysis, the LC-NMR method has the following features:

Advantages:

1. The LC-NMR method is a non-destructive method. Compounds covering a wide range of polarities can be analyzed. The NMR detector is a universal detector that provides a maximum of structural information including information on structure isomers.
2. Complete chromatographic separation, which is often difficult with complex environmental samples, is no prerequisite. As a result of the very narrow NMR signals, in many cases several compounds can be identified within a single spectrum.
3. The NMR detector more directly reflects the concentration of a component in the sample. Quantification of an identified compound can be performed in the on-flow mode using a freely selectable standard. This is advantageous in those cases in which no reference compounds are commercially available (e.g. for metabolites or other transformation products).

Disadvantages:

1. Compared to other methods LC-NMR is still relatively insensitive (at 600 MHz the detection limit in the on-flow mode is in the lower ppb range and in the stopped-flow mode in the ppt range)
2. The NMR detector does not provide direct information on the molecular weight and on hetero atoms.
3. Up to now, LC-NMR has been an expensive analytical tool.

3.4 Examples of application

3.4.1 Detection of explosives and related compounds in groundwater samples of a TNT-contaminated ammunition waste site

As a result of extensive production of ammunition before and during World War II, a large number of hazardous waste sites exist in Germany, the groundwater of which was contaminated by process wastewater, loading and packing facilities, and other military activities. Often the ammunition production sites were located near large groundwater reservoirs, which today, in some instances, represent important drinking water reservoirs. This underlines why non-target analysis of such ammunition waste sites is of particular importance. Studies by us and others [1–3] have revealed that aqueous samples from such ammunition waste sites may also contain a variety of highly polar compounds. Figure 1a shows the UV chromatogram, Figure 1b the on-flow NMR chromatogram of a groundwater sample from the former ammunition production site in Stadtallendorf (Hesse,

Germany). One axis represents the retention time, the other the chemical shift. The big advantage of NMR chromatograms is their high selectivity. For instance, the peak at 87.92 min in the UV chromatogram is formed by the main components 5-hydroxy-2,4-dinitrobenzoic acid, 2-nitrobenzoic acid, and benzoic acid, as well as by the minor components 2-nitrobenzyl alcohol and 4-nitrobenzyl alcohol, in which the main components completely co-elute. The limit of detection in the NMR chromatogram under the applied conditions and after SPE extraction was in the lower µg range absolute. Less polar explosives were removed beforehand by pre-extraction. In this sample, many unknown polar components could be identified. Wherever components could not be identified by comparison with a multi-standard, the MS and NMR data generated on-line were used to make initial structure proposals, which were later on, confirmed by comparison with authentic reference materials. This is demonstrated in Figure 2, which shows extracted rows of the NMR chromatogram in Figure 1. The MS data generated for these compounds provided information on the quasi-molecular ion $[M\text{-}H]^-$ at m/z = 180 and on the presence of a carboxylic group (m/z = 136, $[M\text{-}H\text{-}CO_2]^-$) and a nitro group (m/z = 106, $[M\text{-}H\text{-}NO\text{-}CO_2]^-$) in the molecule, while the NMR spectra enabled recognition of the methyl group and the substitution pattern at the aromatic ring. Based on the NMR and MS data, the compounds could preliminarily be identified as methylnitrobenzoic acids, which were later on confirmed by authentic reference compounds.

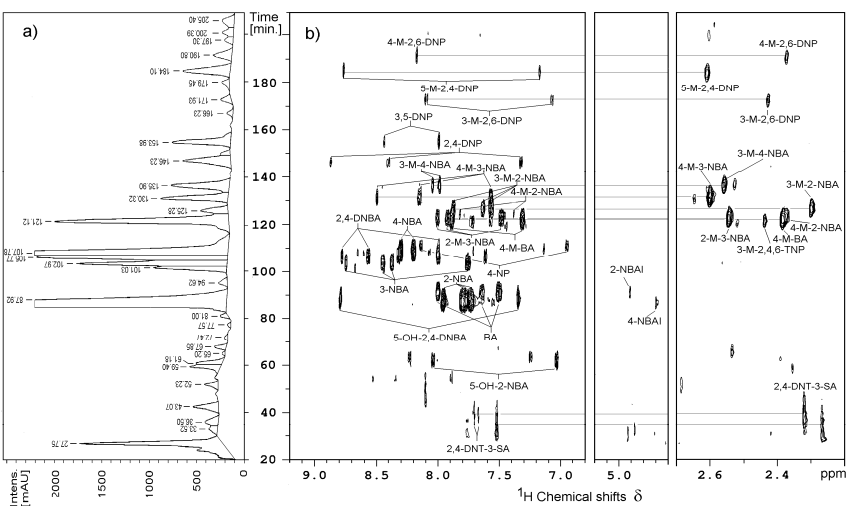


Figure 1: Extract of a groundwater sample from a TNT-contaminated waste site in Stadtallendorf (Hesse, Germany). (a) HPLC chromatogram at 230 nm (b) NMR chromatogram. Reprinted from reference [4] with permission from Analytical Bioanalytical Chemistry.

In cases where ^{1}H NMR and MS data generated on-line were insufficient to make well-founded structure suggestions, compounds were isolated by HPLC fractionation and elucidated by further 2D NMR and MS^n investigations. This can be necessary, for instance, for highly substituted or highly symmetrical compounds.

As an example the structure elucidation of a highly substituted unknown transformation product of TNT is discussed. From the LC-NMR data of this compound it could be concluded that it must be a tetra-substituted aromatic compound with two protons in para-position. MS/MS data prove the presence of one carboxylic and two nitro groups in the molecule, MS data recorded in D_2O the presence of two exchangeable protons, and high-resolution MS data the empirical formula $C_7H_4N_2O_7$. From these data it could be concluded that the unknown compound must be a hydroxydinitrobenzoic acid. Unfortunately, there are 16 possible constitution isomers, and an identification of this compound as 5-hydroxy-2,4-dinitrobenzoic acid was only possible on the basis of the 2D heteronuclear multiple bound correlation (HMBC) NMR spectrum. Spectroscopic data are summarized in Table 2.

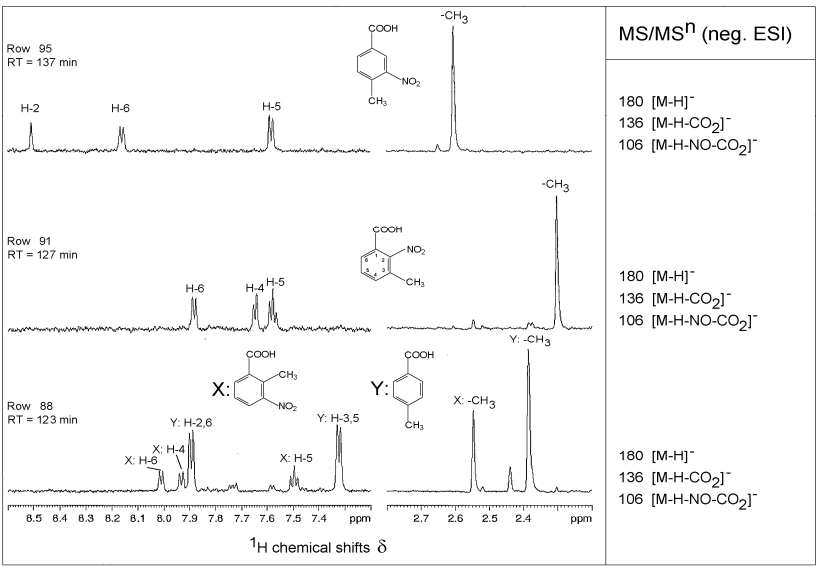


Figure 2: Extracted rows from the NMR chromatogram in Figure 1 (left-hand side) and LC-MS data (right-hand side). The extracted rows show the ^{1}H NMR spectra of some methyl-nitrobenzoic acids (meta-couplings are not resolved, RT retention times). NMR chromatogram reprinted from reference [4] with permission from Analytical Bioanalytical Chemistry.

Table 2: NMR and MS data of 5-hydroxy-2,4-dinitrobenzoic acid.

Chem. shifts δ [ppm]		MS/MS^n (ESI neg.)		HR-MS
^{1}H	^{13}C	H_2O	D_2O	H_2O
H-3: 8.73 (s) H-6: 7.28 (s)	C-1: 139.2 C-2: 137.5 C-3: 124.3 C-4: 135.4 C-5: 158.6 C-6: 120.4 C-7: 168.8	MS: 227 $[M-H]^-$ MS^2: 183 $[227-CO_2]^-$ 153 $[227-CO_2-NO]^-$ 123 $[227-CO_2-N_2O_2]^-$	MS: 228 $[M-D]^-$	$[M-H]^-$ calculated: 226.9935 measured: 226.9939

3.4.2 Detection of transformation products of nitramines in groundwater samples from an RDX-contaminated ammunition waste site

The ammunition waste site in Elsnig (Saxony/Germany) is dominated by the nitramine hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and its by-product octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). Figure 3 shows a section of the NMR chromatogram of the groundwater extract. For the first time, hexahydro-1,3-dinitro-5-nitroso-1,3,5-triazine and octahydro-1,3,5-trinitro-7-nitroso-1,3,5,7-tetrazocine, which are degradation products of RDX and HMX, respectively, could be detected on the basis of their LC-NMR data in a real groundwater sample [5].

3.4.3 Detection of unusual biodegradation products in groundwater samples from an MNT-contaminated ammunition waste site

The last application deals with the identification of unusual polar metabolites in the aquifer of an ammunition waste site contaminated by large amounts of mononitrotoluenes (MNT's).

Mononitrotoluenes result from the first nitration step of TNT production and were discharged into the environment probably due to damage of the TNT production line during World War II. The NMR chromatogram of the groundwater extract is shown in Figure 4. As main polar components besides 4-aminobenzoic acid three derivatives of quinolinyl-3-acetic acid could be identified from the LC-NMR and LC-MS data (Fig. 5). In the context of explosives and related compounds these are very unusual metabolites. In the meantime, evidence could be provided by microcosm experiments with soil material from the waste site that these metabolites are really microbial transformation products of the mononitrotoluenes.

The question of how these metabolites were formed is not easy to answer. Possibly, a mechanism that has been discussed in the literature for the formation of benzoic acid and methylbenzoic acids from BTX [6] under anaerobic conditions (see Figure 6) could be an explanation. Assuming this mechanism works also in the biodegradation of o-toluidine, formation of the identified derivatives of quinolinonyl acetic acid could be explained by intra-molecular cyclization of the corresponding intermediates as shown in Figure 7.

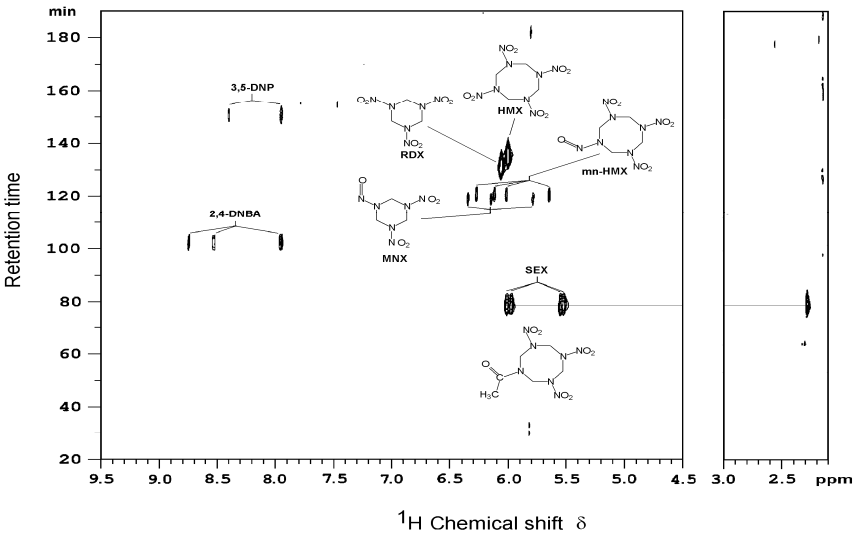


Figure 3: NMR chromatogram of a groundwater extract from an RDX-contaminated waste site in Elsnig (Saxony, Germany). Besides the nitramines the degradation products MNX and mn-HMX could be identified.

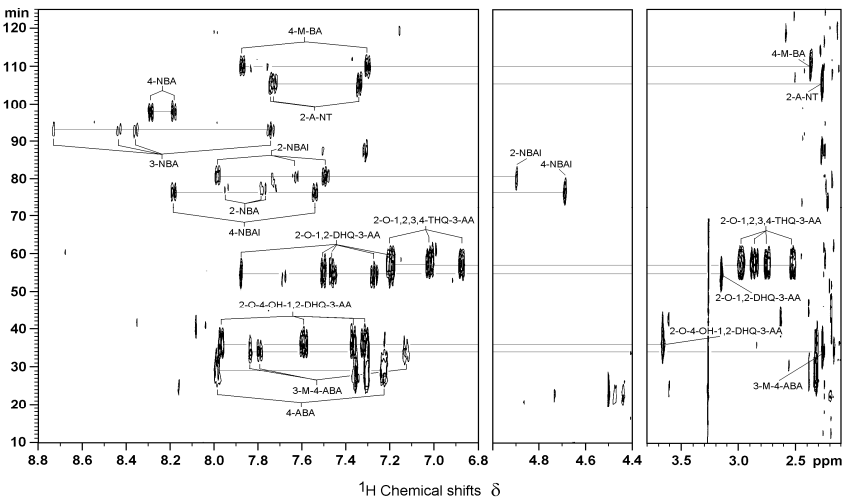
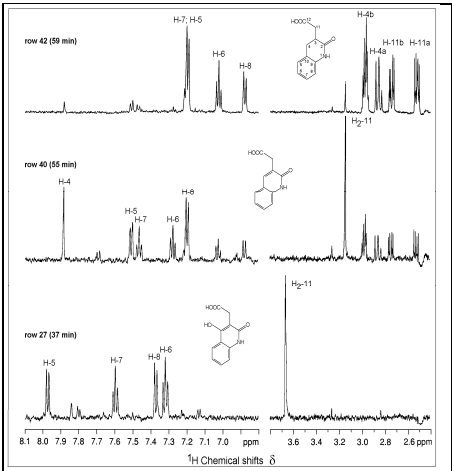


Figure 4: NMR chromatogram of a groundwater extract from the MNT-contaminated ammunition waste site Kleinniederung (Hesse, Germany).



MS/MS^n	
ESI pos.	ESI neg.
206 $[M+H]^+$ 188 $[M+H-H_2O]^+$	204 $[M-H]^-$ 160 $[M-H-CO_2]^-$
204 $[M+H]^+$ 186 $[M+H-H_2O]^+$	202$[M-H]^-$ 158 $[M-H-CO_2]^-$
220 $[M+H]^+$ 202 $[M+H-H_2O]^+$	218 $[M-H]^-$ 174 $[M-H-CO_2]^-$

Figure 5: Extracted rows from the NMR chromatogram in Figure 4 (left-hand side) and LC-MS data (right-hand side). The extracted rows show the ^{1}H NMR spectra of the identified unusual metabolites.

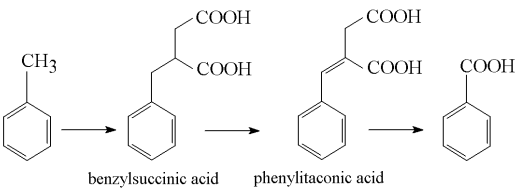


Figure 6: Proposed pathway and metabolites for the anaerobic degradation of toluene [6].

4 Conclusions

It was shown that polar contaminants in groundwater can be characterized (after pre-concentration) in the lower ppb range by the combined use of LC-NMR and LC-MS. On-line identification of unknowns (non-target analysis) can be achieved in many cases; in other cases, valuable structural information on the pollutants and their transformation products can be obtained. On the basis of LC-NMR and LC-MS results, simpler and less expensive methods may be developed in a second step for target analysis of important identified pollutants or metabolites.

At present, applications of LC-NMR in environmental analysis are still limited by the relatively low sensitivity of the NMR detector. However, an increase in sensitivity of cryoprobes which are now also available as flow probes for instruments up to 600 MHz and the application of repetitive SPE trapping will probably further promote the use of LC-NMR for comprehensive characterization of environmental samples.

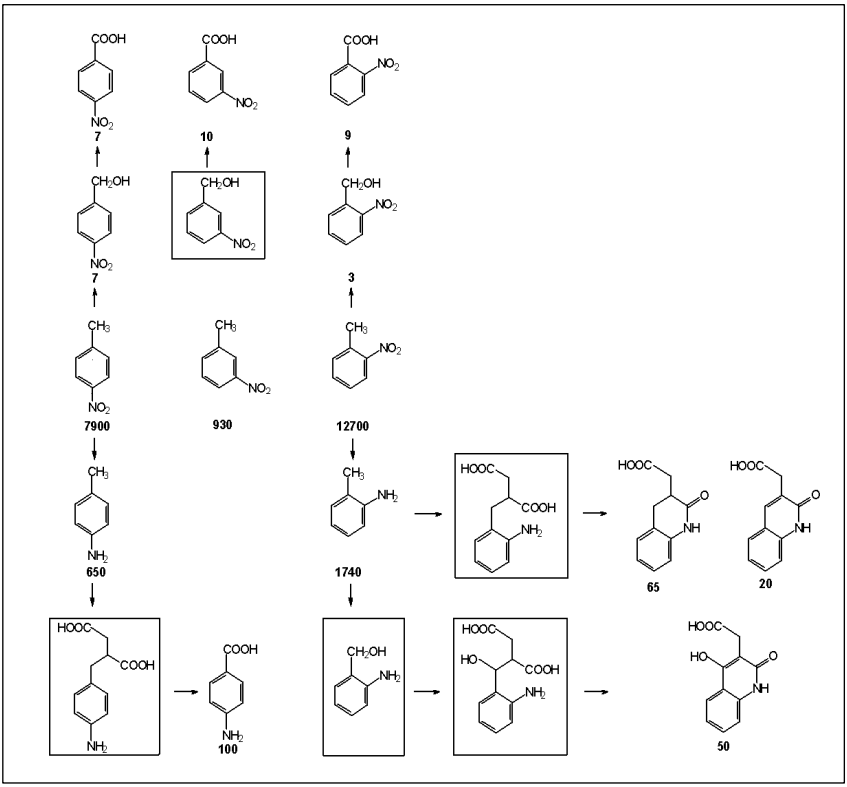


Figure 7: Transformation products of mononitrotoluenes. Structures in the frames could not be detected and are postulated, number below the formula indicate the determined concentration [µg/L].

Acknowledgement

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References

[1] Preiss A., Lewin U., Wennrich L., Findeisen M.& Efer J., Analysis of nitrophenols and other polar nitroaromatic compounds in ammunition wastewater by high-field proton magnetic resonance (^{1}H-NMR) spectroscopy and chromatographic methods. *Fresenius Journal Analytical Chemistry,* 357 (6), pp. 676–683, 1997

[2] Godejohann M., Astratov M., Preiss A., Levsen K. & Mügge C, Application of continuous-flow HPLC - proton-nuclear magnetic resonance spectroscopy

for structural elucidation of phototransformation products of 2,4,6-trinitrotoluene Analytical *Chemistry,* 70 (4), 4104–4110, 1998

[3] Schmidt T.C., Steinbach K., v. Löw E. & Stork G., Highly polar metabolites of nitroaromatic compounds in ammunition wastewater, Chemosphere 37(6), 1079–10990, 1998

[4] Preiss A., Elend M., Gerling S., Berger-Preiss E.& Steinbach K., Identification of highly polar nitroaromatic compounds in leachate and ground water samples from a TNT-contaminated waste site by LC-MS, LC-NMR, and off-line NMR and MS investigations, *Analytical & Bioanalytical Chemistry*, 389(6), 1979–1988, 2007

[5] Preiss A., Elend M., Gerling S., & Träckner S., Analysis of highly polar compounds in groundwater samples from ammunition waste sites. Part I – Chararacterization of the pollutant spectrum, *Magnetic Resonance in Chemistry,* 43 (9), 736–746, 2005

[6] Griebler Ch., Safinowski M., Vieth A., Richnow H. H., & Meckenstock, R.U., Combined application of stable carbon isotope analysis and specific metabolites determination for assessing in situ degradation of aromatic hydrocarbons in a tar oil-contaminated aquifer, Environmental *Science &Technology*, 38(2) 617–631, 2004