Final report

TN Interlaboratory Study in Summer 2002

This interlaboratory study was performed within the project :
Verbesserte Bestimmung des Gesamtstickstoffgehalts in Süßwasser-, Brackwasser- und Seewasserproben - Validierung der Analyseverfahren

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1 Introduction

Total nitrogen (TN) is one of the key parameters to describe the environmental status of aquatic ecosystems. This parameter is included in most national and international monitoring programs and determined by a large number of laboratories. For the comparability in monitoring programs it is essential that the results produced by different laboratories are comparable. To achieve this, different quality assurance systems were established on laboratory, national and international scale. One point in this quality assurance system are interlaboratory studies between laboratories, which should prove that the different methods or modifications of methods applied will give the same results.

In aquatic systems, TN includes a broad variety of different compounds ranging from 'simple' inorganic nutrient salts (nitrate, nitrite, ammonia) to complex organic molecules in which nitrogen is bound in different structures. Due to this complex composition of TN there is no method known to measure TN concentration directly. All methods published require the complete conversion of all different N – containing compounds in a sample into a specific reaction product which can be determined specifically. According to the reaction used for the digestion step and the resulting product, methods for the determination of TN can be separated into three basic groups (Fig. 1).

The Kjeldahl method, which is known for more than 100 years (Kjeldahl (1883)), is nowadays not commonly used for analysis of environmental samples because it is laborious to perform and gives many chances to produce uncertain results. Some 40 years ago, wet digestion methods were published which are more applicable for routine use and to analyze larger numbers of aquatic samples. One method widely used in the last years is that published by Koroleff (Koroleff (1983)). Since the middle of the 1980 catalytic oxidation methods were adopted to assay TN or DN in aquatic samples (Suzuki et al. (1985)). One advantage of catalytic oxidation methods is that they are easy to perform. For all methods numerous modifications are published, mainly because there are erroneous results found under specific conditions or in some special samples.

Although TN determinations were performed by many laboratories for some years in monitoring programs, the 'community precision' for this parameter is significantly lower than that achieved for other determinants, e.g. for most of the inorganic nutrient salts. It can be contributed to the complexity of the methods used for TN determination that results reported in interlaboratory studies show higher deviation due to relatively small variations occurring in single steps of the procedure. Apart from external quality assurance systems, the internal use of reference or control material in every analysis is recommended. However, this recommendation caused some problems in the past, because appropriate (natural) reference material had not been available. The use of artificial reference or control material may not always be the first choice, as it is known for some procedures

that artificial material does not prevent erroneous results caused by matrix effects (for example see Arminot & Kérouel (2001)).

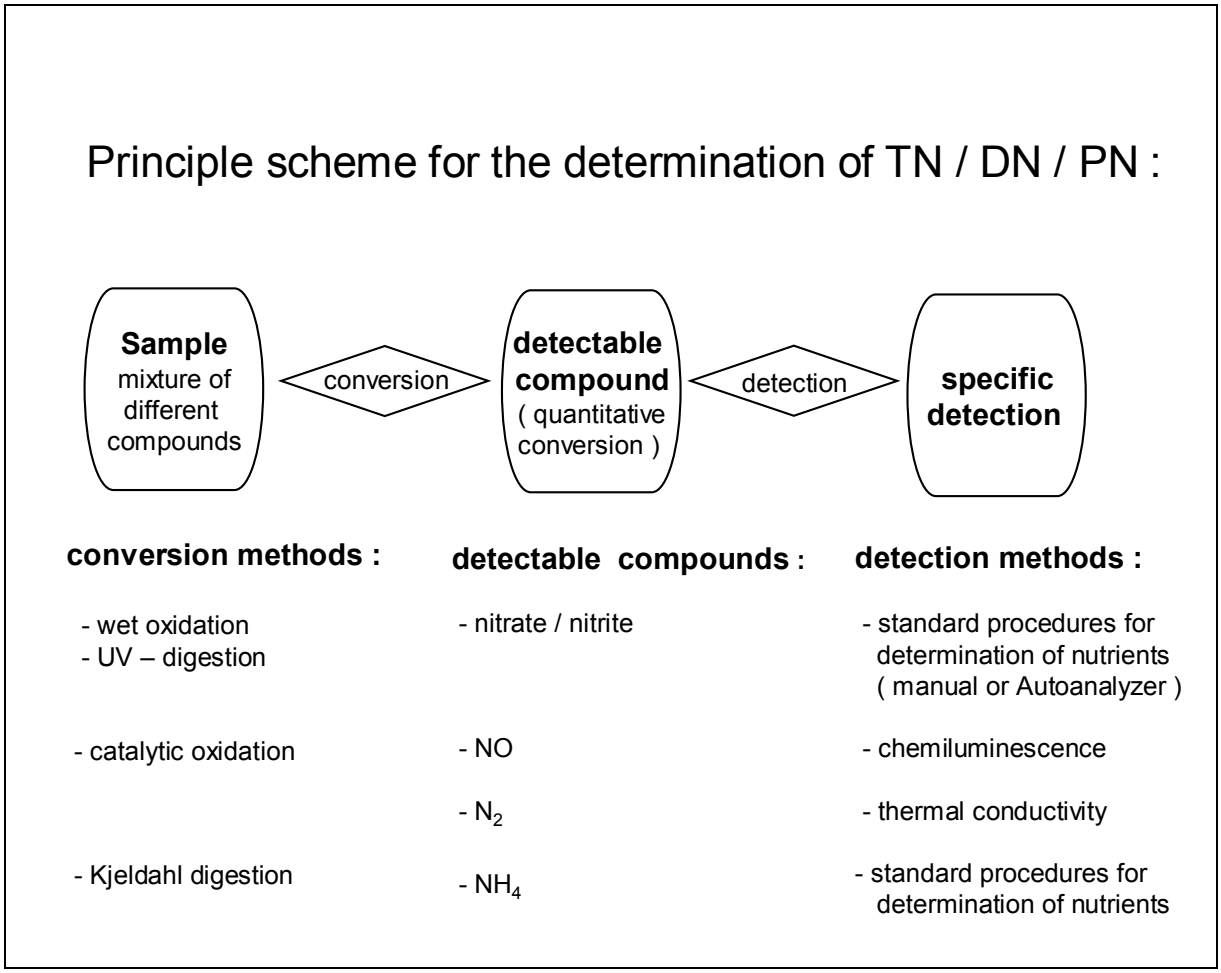


Fig. 1 Classification of methods applied for the quantification of TN (and DN & PN) in aquatic systems.

Since one or two years however, some natural reference materials are offered for inorganic nutrient salts, TN (DN) and TP (DP) by QUASIMEME (QUASIMEME; http://www.quasimeme.marlab.ac.uk/) or for DOC (and DN) (Hansell (2001),). Although it may be difficult or expensive to obtain this reference material, its broad use can significantly enhance the 'community precision' in interlaboratory studies and the reliability of results.

2 Sample Material

2.1 Selection of Sample Material

Samples analyzed by the laboratories involved in the BLMP ranged from samples taken in inshore lakes and rivers to samples taken in the marine ecosystems and cover a broad range of concentrations and different matrices. Therefore a number of different samples had been taken for this interlaboratory study from inshore lakes and some coastal and offshore stations in the Baltic Sea area during the years 2000 to the beginning of 2002. Three of these samples had been selected for this exercise differing in concentrations of TN and / or salinity. Additionally a fourth sample was added, which was prepared synthetically by dissolving pure Creatinin in pure water (Milli-Q plus water). This sample was added to improve the completeness of the digestion step of the methods used by the participating laboratories.

Although it is stated that total nitrogen (TN) should be determined, filtered samples are used for the natural samples in this interlaboratory study. So strictly speaking this exercise is an interlaboratory study of total dissolved nitrogen (DN). The filtration step is included because it is hardly possible to guarantee the stability and homogeneity of unfiltered samples due to a number of particle forming and degrading processes taking place during the storage process and because of adsorption effects at the walls of the sample containers. This procedure to filter the samples (at 0.2 µm) is also applied by QUASIMEME for the preparation of TN reference material, which is available since the second half of 2002 (see : Information on aqueous test materials for nutrients; http://www.quasimeme.marlab.ac.uk/).

2.2 Preparation of Sample Material

Natural samples were filtered immediately after sampling using Whatman GF/F filters and a vacuum filtration system with controlled vacuum and - depending on the volume of the sample - were transferred to acid cleaned 2.5 l glass bottles or preconditioned and acid cleaned 5 l or 10 l plastic (LDPE) carboys. The initial concentrations of nutrient salts and organic material and the salinity is shown in Annex Tab. A3.
In the tightly closed containers, samples were aged for 4 to 6 months in a dark cooling chamber (+8°C) to allow decomposition of labile material and oxidation of reduced compounds. Container were not opened until the samples were transferred into the ampoules used for storing and distributing the samples. Initial concentrations of nutrient salts, organic material and salinity are given in Annex Tab A3. It is known from other TN or DN interlaboratory studies that estuarine or marine samples are stable for far more than a year, even if they are stored at room temperature in the dark (Sharp et al. 2002b).

For the interlaboratory study, aliquots of each sample were transferred to 50 ml, 20 ml or 10 ml glass ampoules that had been rendered 'organic free' by a procedure which includes incubation in 10% HCL overnight, rinsing thoroughly with Milli-Q water and baking in an oven at 450°C for 6 hours. The ampoules were filled according to the procedure described by Sharp et al (2002a) and sealed immediately using a portable propane torch. No acid or preservatives were added to the samples.

As in the sample taken from an inshore Lake in Thüringen (Sample B in this interlaboratory study) organic material precipitated during the aging period, this sample is filtered a second time and stored in a tightly closed carboy in the cooling chamber for an additional aging period of about two months. When the samples were transferred to ampoules afterwards, no particles were visible. Precipitation of brownish organic material during the aging period does not only appear in this lake sample used for this interlaboratory study, but also in samples taken from other inshore lakes. It is assumed that this precipitate is caused by high amounts of relatively labile humic substances in the lake waters that tend to form precipitates during the aging process.

The fourth sample was prepared synthetically by dissolving Creatinin (Merck No 105206, für biochemische Zwecke) in pure water (Milli-Q plus water). Before transferring to ampoules, the sample was stored in a glass container for some days in a refrigerator (4°C – 8°C). Concentrations of nutrient salts and organic material in the samples after filling the ampoules are provided in Annex Tab. A4.

2.3 Preparation of ampoules

The samples were transferred to ampoules several weeks before sending them out by the procedure described elsewhere (Sharp et al. 2002a). Each filled ampoule was numbered individually so that the sequence and time of filling can be retraced. After sealing the ampoules were stored in a cooling chamber (+8°C) in the dark. The ampoules send out to the individual laboratories were selected randomly from the whole set of ampoules of a sample. This procedure was chosen in order to detect possible systematic errors during the filling process and to check the stability and homogeneity of the whole set of ampoules prepared for each distributed sample.
According to the requests of the laboratories, ampoules with appropriate sample volume were send out at the beginning of June 2002 and arrived within 24 hours in the participating laboratories.

2.4 Stability and homogeneity check

As sample containers were not opened during the aging process, generally not concentrations had been measured during the aging time. Only for sample A which was taken as a large volume sample on a monitoring cruise in 2001, a sub sample was

separated after the filtration step and aged separately. This sub sample was used for other purposes and analyzed in May 2001, about three months after sampling. The results of these measurements are shown in Fig. 2 together with those obtained for the ampoules filled from the main batch of this sample.

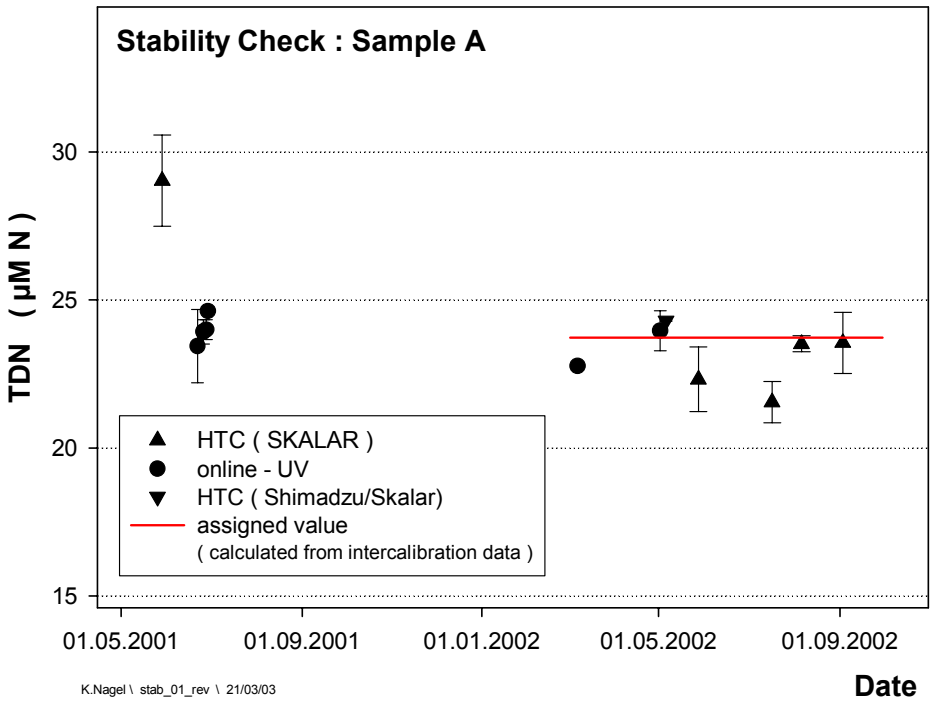
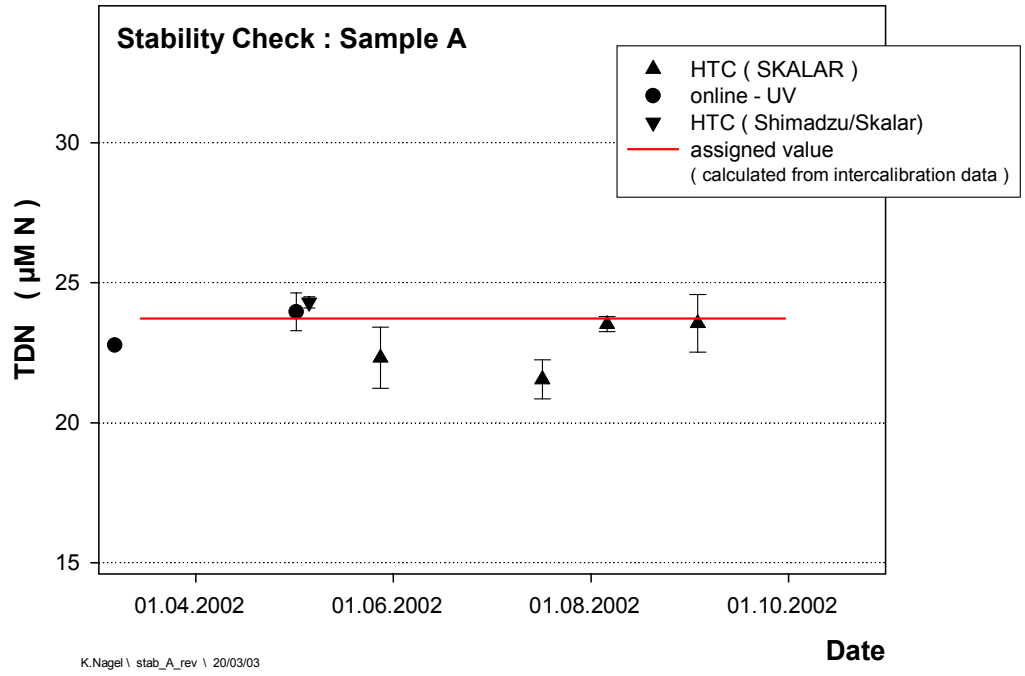


Fig. 2 : Stability check of Sample A
Samples analyzed in May 2001 and in summer 2002 had been aged in two separate containers !

After transferring the samples into the ampoules, from each sample used for this interlaboratory study a set of ampoules was selected to check the stability and homogeneity at different times between the filling of ampoules and the end of data submission. For each check two or three ampoules were selected which were filled near the beginning and near the end of the filling procedure of each sample. In the case of the analysis of 3 samples, the third sample was selected randomly from those ampoules filled around the middle of the filling process. The analyses of these ampoules was performed by HTC – methods as well as with the UV – online method. The results are given in Fig. 3.

Although there are some deviations among the measurements, no systematic concentration change can be proven. There are no hints from these measurements that there are inhomogeneities or systematic errors caused by the filling process.



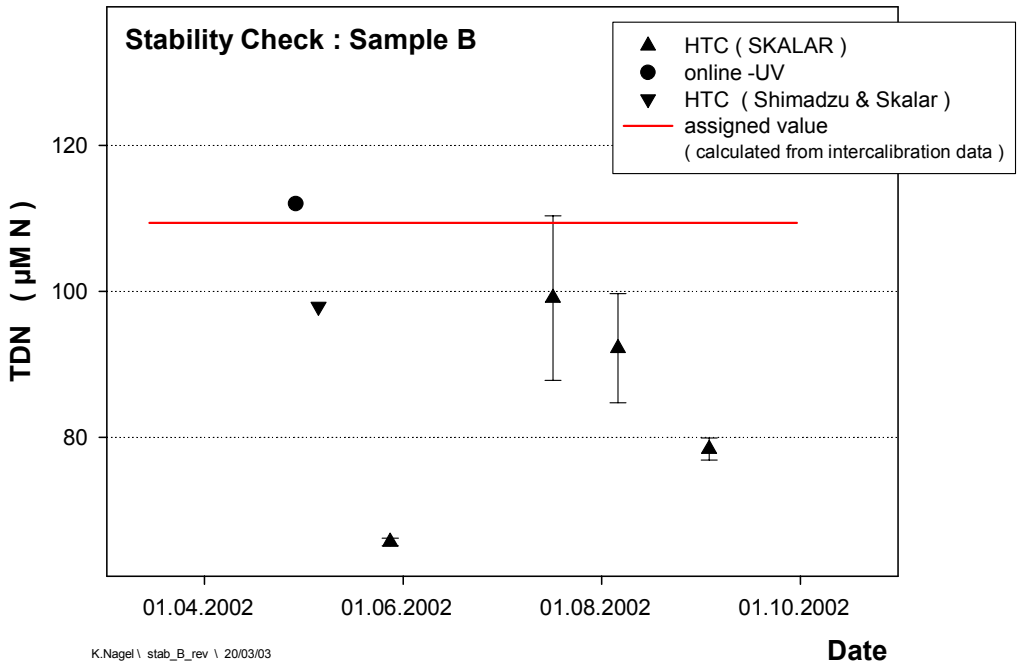
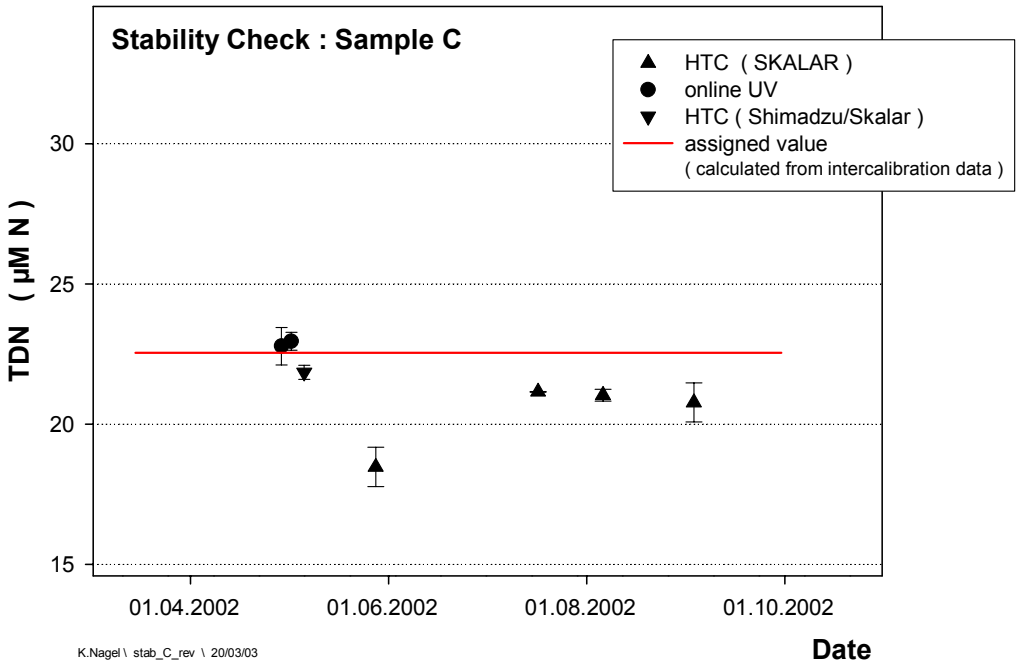


Fig. 3 : Stability of the samples used for TN interlaboratory study during summer 2002



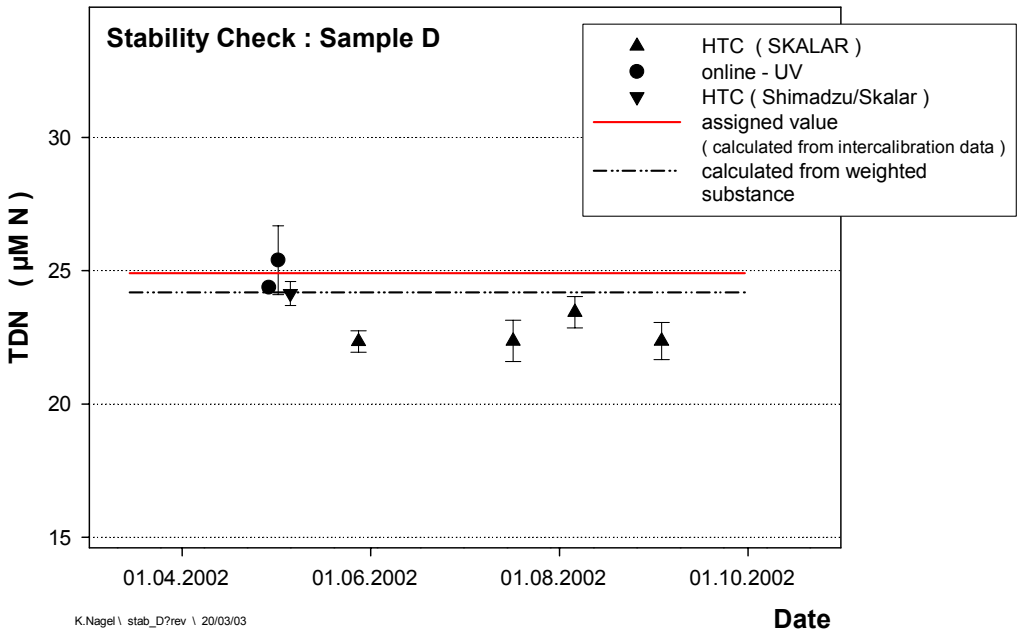


Fig. 3 (continued)

3. Interlaboratory study

3.1 Time schedule and participants

This interlaboratory study was organized by the Institut für Ostseeforschung Warnemünde within the project 'Verbesserte Bestimmung des Gesamtstickstoffgehalts in Süßwasser-, Brackwasser- und Meerwasserproben – Validierung von Analyseverfahren', which is supported by the Umweltbundesamt Berlin (Project No 200 22 231). Samples used for this interlaboratory study were partly taken within regular activities of the institute, partly within the project during the years 2000 – 2002 and filled into ampoules in early spring 2002. The ampoules were sent out to the laboratories between 03/03/02 and 05/06/02 by express mail and arrived in almost all laboratories within 24 hours.

Laboratories were asked to report the results until the beginning of August, but the last results did not arrive before the mid of September 2002. Reported data were checked for consistency and formatted in Excel worksheets for the first evaluation process. The evaluation of the results according to DIN ISO 5725 with the program PROLAB98 (Dr. Uhlig, quo data, Dresden) was performed by Dr. P. Woitke and Dipl.-Ing J. Wellmitz (FG II 3.5, Umweltbundesamt Berlin). This first evaluation step was finished at the end of September. After analysis of the outcome it was decided to exclude some samples and reevaluate the results. This step was finished in November.

Samples had been sent out to 22 laboratories (plus the laboratory of the organizing institute). One laboratory draw back his attendance to participate because of organizational reasons. Laboratories reporting results are listened in Annex Tab. A1. As some of the laboratories reported results obtained with more than one method, the number of data sets available for the evaluation was 29. It should be noticed that an individual 'Laboratory Code' was given to every data set reported for measurements performed with one method by a laboratory. The participating laboratories were informed about the laboratory codes assigned to the individual methods.

3.2 Methods applied by the laboratories participating in the interlaboratory study in summer 2002

As all laboratories were asked to apply their 'standard method' for the determination of TN, a number of different methods had been used to obtain the reported results. These methods can be grouped into three major classes with respect to the digestion step of the procedure :

- digestion of the sample in the presence of an oxidant at elevated temperature ('Koroleff' digestion) (16 data sets)
  reaction product to be quantified : nitrate

- digestion of the sample by UV – irradiation (in the presence of an oxidant) (2 data sets)
  reaction product to be quantified : nitrate
- high temperature combustion of the sample in the presence of a catalyst (HTC method) (11 data sets)
  reaction product to be quantified : NO

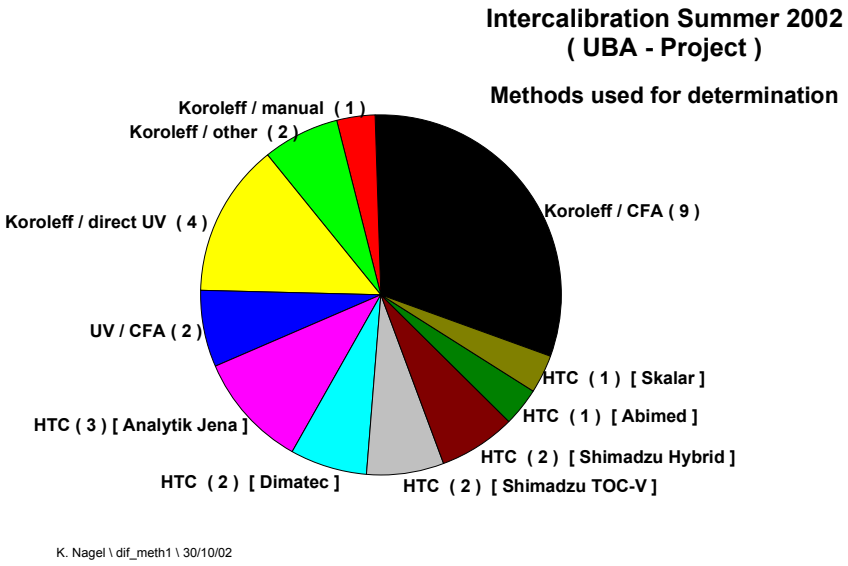


Fig. 4 : Classification of determination methods used in the intercalibration exerecise

Further subclasses can be defined by taking into account the method for the quantification of the reaction products of the digestion step of the 'Koroleff digestion' procedures or by differences in the technical procedures to perform the HTC – methods (Fig. 4).
The number of measurements per sample reported differs between laboratories. Most of them report a duplicate measurement for each sample, others report more than 10 values for the same sample (e.g. single injections in HTC methods). Some of the laboratories got additional ampoules because of damage during transport or due to other reasons. Therefore some laboratories reported results obtained from measurements using a larger number of ampoules of the same sample.

3.3 Reported results and evaluation

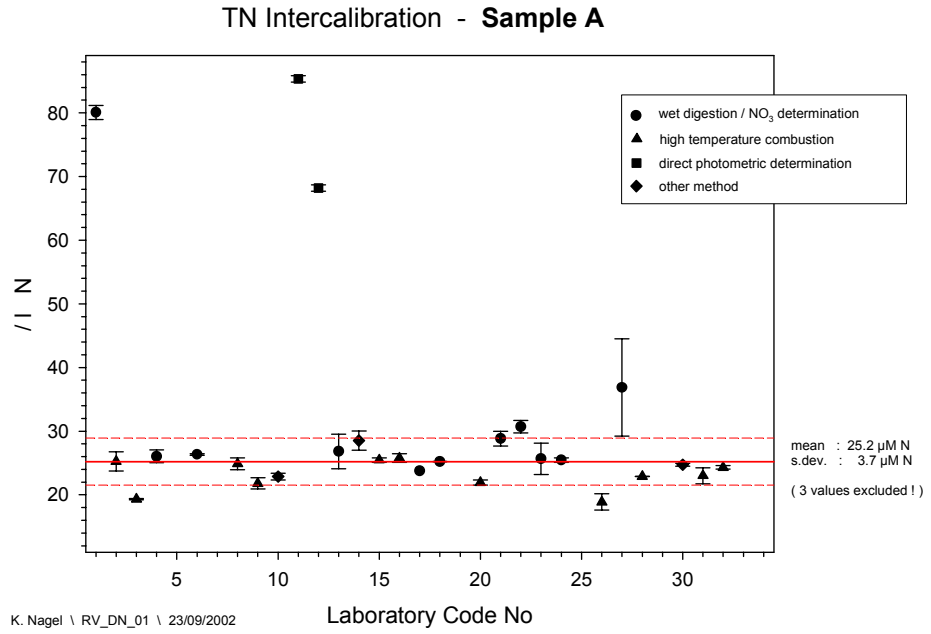
For a first overview, mean values and standard deviations were calculated for each sample and each laboratory. These values are plotted versus laboratory code number and shown in Fig. 5 and are listed as means & s.dev. in Annex Tab. A2. There was no further statistical treatment of the data at this step. It should be noticed, that those data stated as insufficient for different reasons by a laboratory itself were not included. One laboratory reported data obtained with a method which is in a 'testing phase' for this laboratory. As these data seem to be accurate they are included here.

During the second stage all data sets including all reported values for repeated measurements of the same sample are compiled and evaluated according to ISO 5725 using the program PROLAB (Dr. Uhlig, quo data, Dresden). This evaluation step was done by Dr. P. Woitke and Dipl.-Ing. J. Wellmitz (FG II 3.5, Umweltbundesamt Berlin). Only data clearly labelled by the reporting laboratory as insufficient were not used for this evaluation purpose.

The elimination of outliers was carried out according to ISO 5725:
Type A: individual within-laboratory outlier (e.g. one out of four results deviates significantly)
Type B: between-laboratory outlier due to significant deviation of the laboratory mean from the total mean
Type C: between-laboratory outlier due to significant deviation of the within-laboratory standard deviation from the total mean of the within-laboratory standard deviation

Because the values for sample A reported by laboratories 1, 11 and 12 were not eliminated according to ISO 5725 it was decided to exclude these values manually (Type D). After the elimination of outliers the third evaluation of the results for each sample was carried out and shown in Fig. 6. The reasons for samples A and C reported by laboratories 1, 11 and 12 will be discussed in detail later (see. 3.4).

In a fourth step laboratories were grouped according to the methods they used for the determination of TN. The first group includes those laboratories applying wet digestion methods of samples ('Koroleff method') and subsequent determination of nitrate, independently from the method used for nitrate determination.



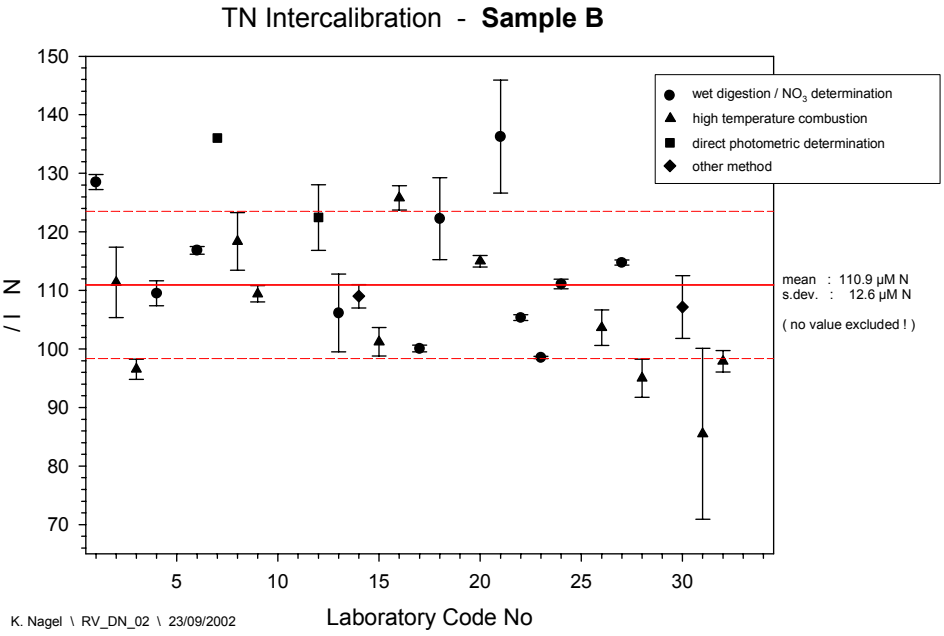
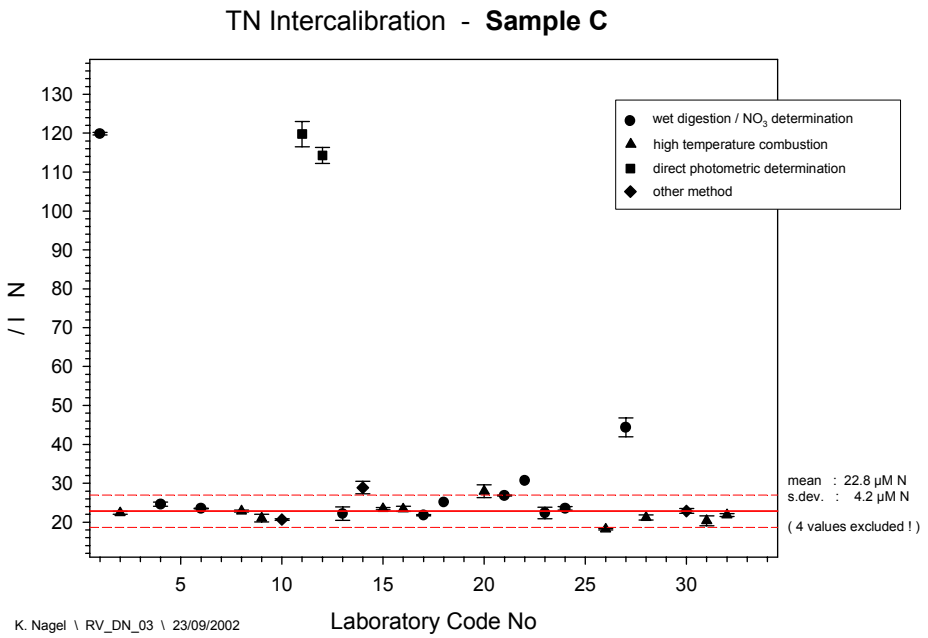


Fig. 5 : Graphs of data reported for TN by the participating laboratories. Data are plotted without statistical treatment.



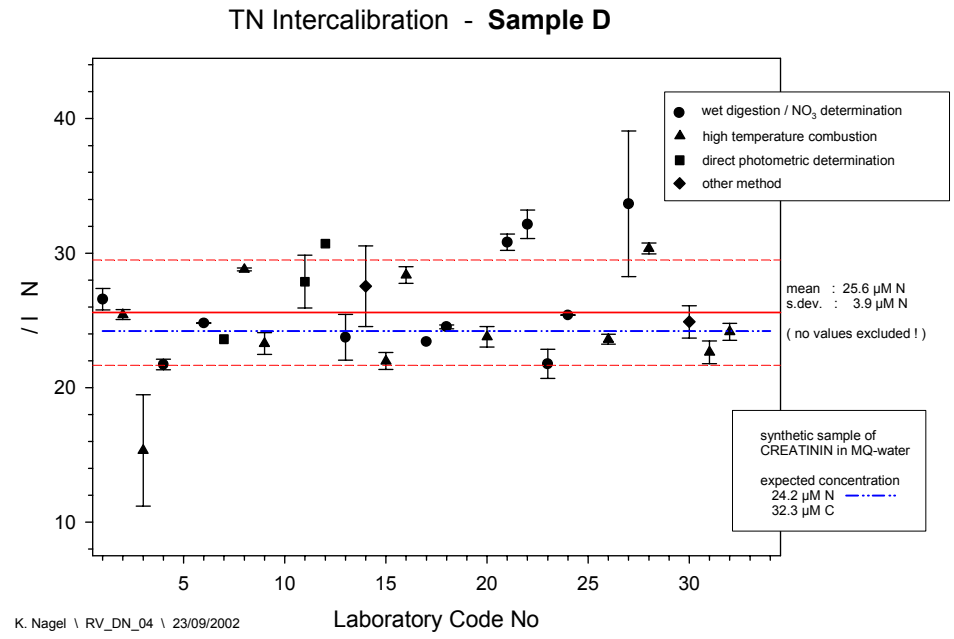


Fig. 5 (continued)

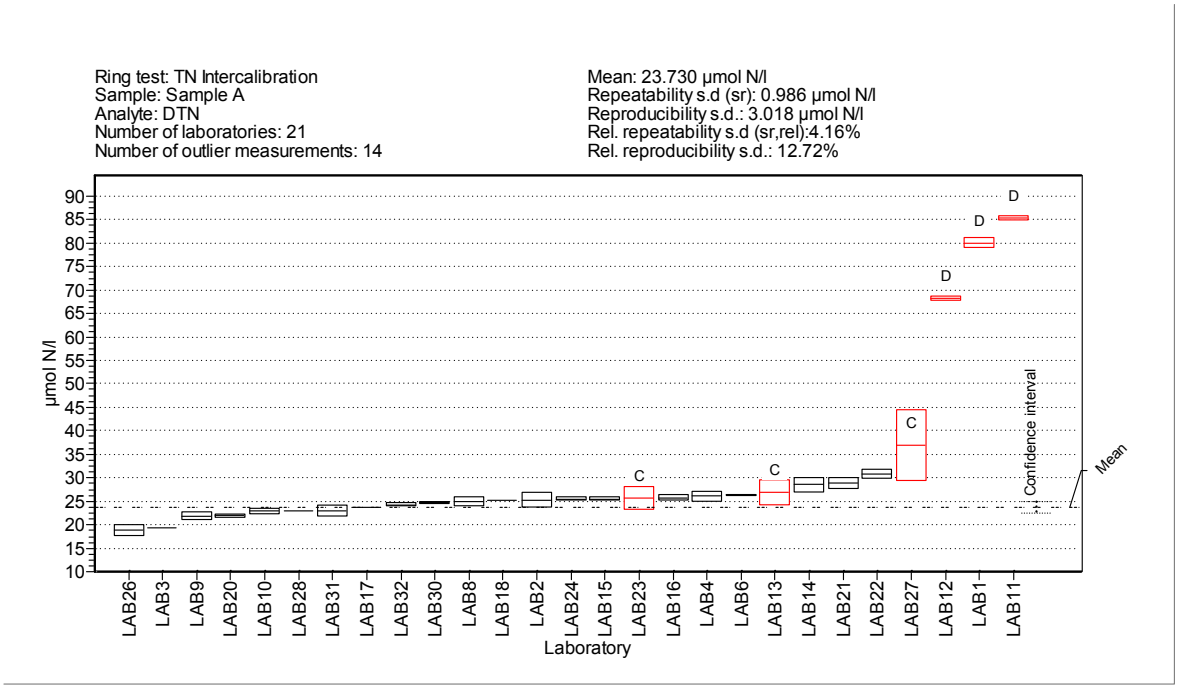
Only 3 laboratories using direct UV determination at 210 nm for nitrate determination (laboratory codes 1, 11, 12) were excluded from the evaluation of samples A and C. In this group 12 (samples A & C) or 16 (samples B & D) are combined. The results of this group is shown in Annex Fig. A1. In another group all laboratories using HTC methods are combined. This group includes 11 laboratories. The results for this group are shown in Annex Fig. A2. Because of the low number of laboratories applying (online-) UV methods this group was not evaluated separately. The results of the second and fourth evaluation step are summarized in Table 1.

3.4 Some remarks on the results of the interlaboratory study

One general outcome of this interlaboratory study is that within this group of laboratories one can expect results 'correct' within a range of approximately 10% to 15%. Although this result is comparable with the outcome of recent other interlaboratory studies (e.g. Woitke et al. (1999), Sharp et al. 2002b), it should not be discussed here whether this result is 'good', 'acceptable' or should give reason to start efforts to improve the 'community precision'. Nevertheless some remarks should be made to some aspects of this exercise.

The first overall view of the results showed extremely high values for the two 'seawater' samples A and C reported by three laboratories (Fig. 5 and Annex Tab.A2). These three laboratories analyzed the samples by wet oxidation step converting nitric compounds to nitrate, which is subsequently quantified by direct UV – measurement at 210 nm. A fourth laboratory, which applied the same technique, did not analyze samples A and C and stated that this method is not suitable for seawater, because of the absorption of chloride and bromide, which are usually present in significant concentrations in seawater. Furthermore, the high amounts of dissolved organic material (DOM) in the samples (documented by high DOC concentrations, see Annex Tab. A3 & A4) also contribute significantly to high absorption in the UV – region. It should be pointed out that two of these laboratories stated to perform their TN determination according to DIN ISO 11905. This ISO standard is suitable also for determination of TN in seawater and provides rules for direct UV determination of nitrate in water showing significant absorption at 210 nm.

During the evaluation process according to DIN ISO 5725, data reported by these three laboratories are eliminated as 'outliers' only for sample C, but not for sample A. As there are some reasons to assume that the 'background absorption' in the samples was not properly corrected for the quantification of nitrate after the digestion step, the data reported for sample A and C by these three laboratories are manually eliminated. The same three laboratories reported inconspicuous results for samples B and D, which are much lower in DOM and salt concentrations.



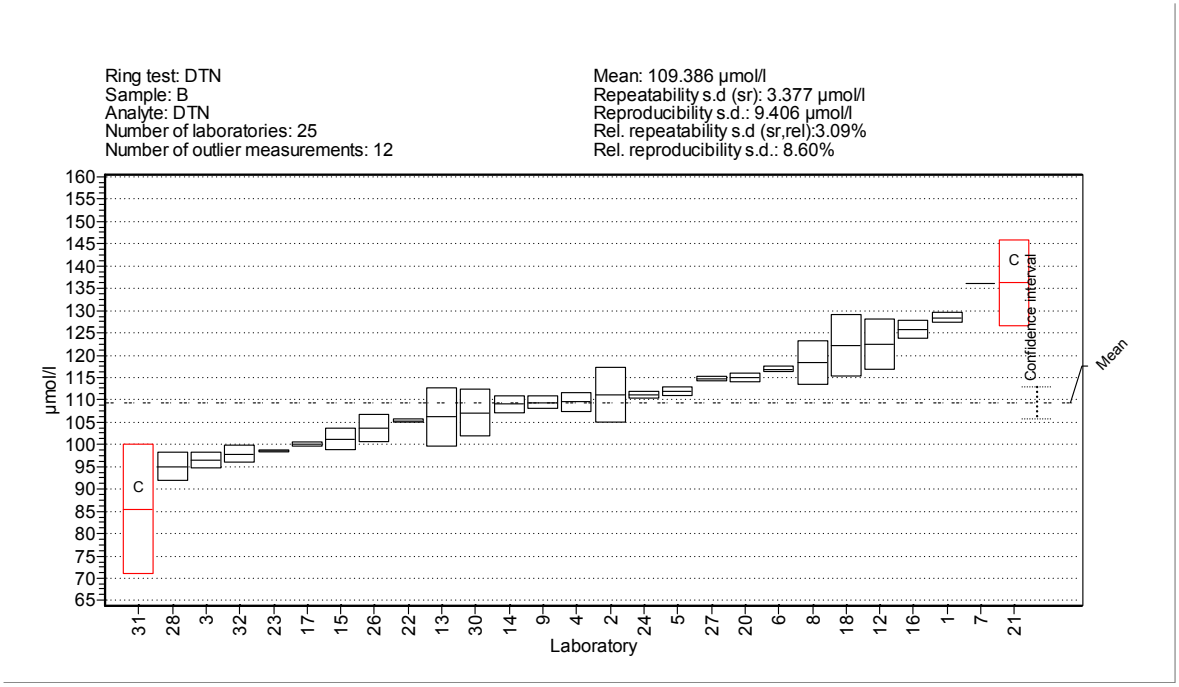
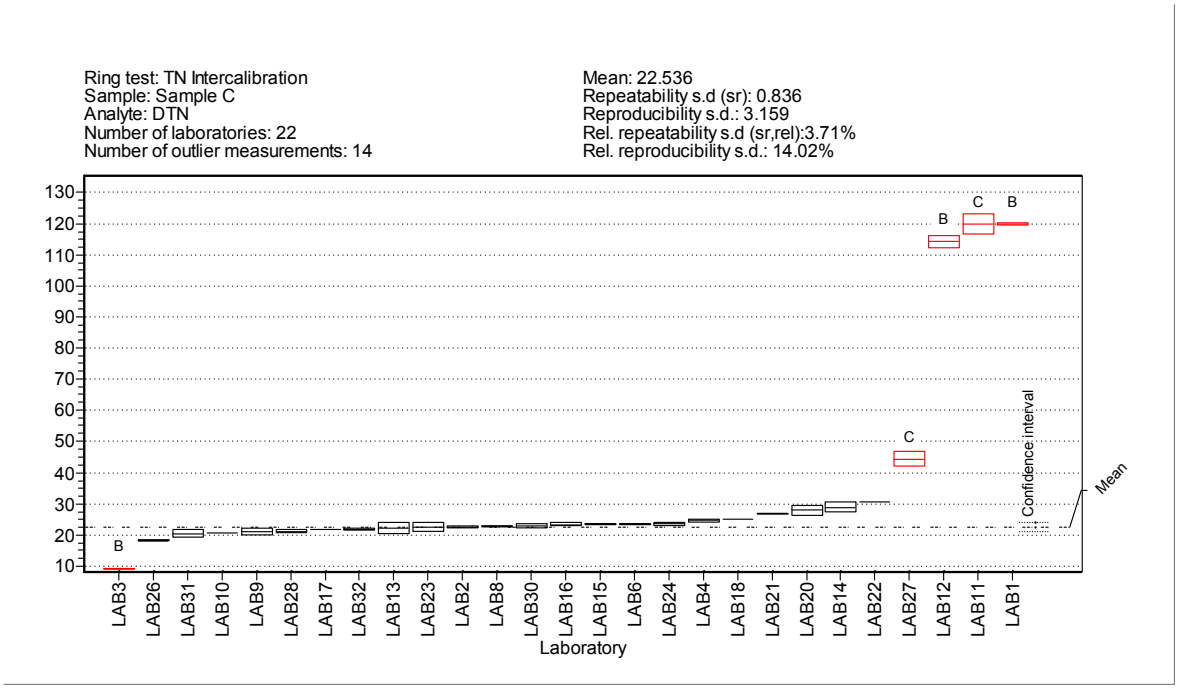


Fig. 6 : Evaluation of the TN – interlaboratory study in summer 2002
Evaluation based on results obtained by all methods !

Types of signed outliers (see also 3.3):
Type B: between-laboratory outlier due to significant deviation of the laboratory mean from the total mean
Type C: between-laboratory outlier due to significant deviation of the within-laboratory standard deviation from the total mean of the within-laboratory standard deviation
Type D: manually determined outliers



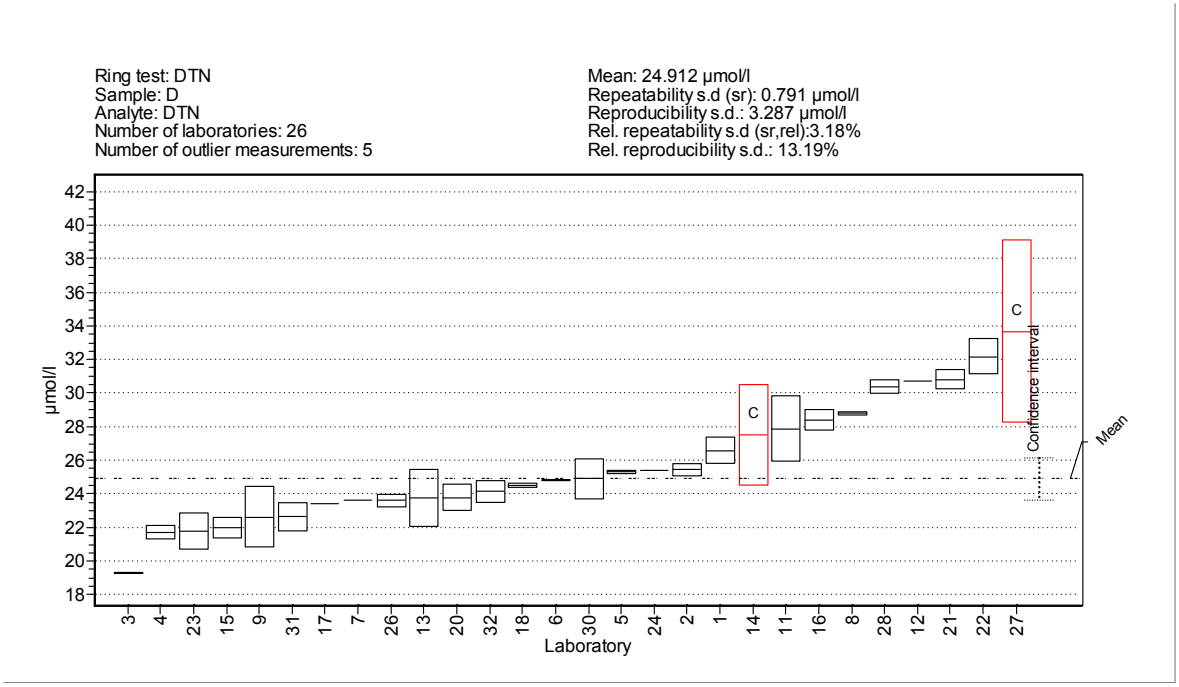


Fig. 6 : (continued)

The methods used by the majority of laboratories participating in this exercise can be grouped into wet digestion according to Koroleff and HTC – methods. Only two laboratories (7%) use an online – UV method on a CFA – system. As the number of laboratories applying this last mentioned method is too low for further statistical analysis and as the results reported by these two laboratories are within the range of reported values, no further comments should be made on this method.

The Koroleff – method or modifications of this method were applied by 16 laboratories (55%). Although the description of the digestion step given by some laboratories is fragmentary, it is obvious that a number of different modifications were used. For the digestion step, this concerns the volume and material of the bottles used, the composition and volume of the reagent added, time and temperature of heating the sample and the 'instrument' used for heating (e.g. autoclave or microwave oven).
9 of these 16 laboratories used CFA – systems to quantify the amount of nitrate produced in the digestion step, one laboratory performed manual nitrate analysis and two laboratories used other methods (Flow-Injection Analysis and Ionic - Chromatography). Four laboratories used direct UV detection of nitrate. Most of the laboratories reported two measurements for each sample, only three or four laboratories reported 3 or 4 numbers per sample (depending on the sample).
The results obtained by this group of laboratories show slightly higher mean values than those calculated for all participants (Tab. 1). The repeatability varies between 0.74 µM N and 1.69 µM N and the reproducibility between 2.54 µM N and 7.77 µM N (Tab 1).

HTC – methods were applied by 11 laboratories (38%) using a very heterogeneous set of instruments. Apart from three laboratories operating a multi N/C 3000 system (Analytik Jena) and two laboratories using a Shimadzu TOC-V system, all others performed their measurements on other (commercially available) instruments. Because of this heterogeneity, which concerns many different chemical and technical aspects (e.g. type of catalyst used, combustion temperature, plumbing of the system) no detailed comparison between different systems should be given here.

As only a rather small sample volume is injected for a single measurement on a HTC system, laboratories using this technique reported a larger number of single values for each sample. In most cases 4 or 6 values were reported, some laboratories submitted even more single results (up to 13 in one case). Due to fragmentary description of the details of the method used, the exact meaning of these numbers in the measuring process of the individual HTC systems is not absolutely clear.
Most HTC systems use a present number of single injections to calculate a mean value and a standard deviation. If the standard deviation exceeds a preset limit, additional injections are performed and – according to the number of additional injections – those values with major contribution to the standard deviation are canceled. This process is controlled automatically by the HTC system and the mean (together with the standard

deviation) calculated according to these preset conditions is the 'measured concentration' in the sample.

Tab. 1 : Compiled results of the TN – interlaboratory study evaluated according to DIN ISO 5725 for TN determination

Results of the evaluation of all reported values - independently from the method used for the analysis – are compared with those obtained by the group of laboratories applying Koroleff – methods or HTC – methods.
In the group applying Koroleff – methods, those laboratories using direct UV – measurements for the determination of nitrate are excluded for samples A & C.
Numbers are taken from Fig. 6 and Annex Fig. A1 and A2.

	Sample A	Sample B	Sample C	Sample D
All Methods				
mean	**23.730**	**109.386**	**22.541**	**24.912**
repeatability	0.986 (4.16%)	3.377 (3.09%)	0.826 (3.66%)	0.791 (3.18%)
reproducibility	3.018 (12.72%)	9.406 (8.60%)	3.088 (13.70%)	3.287 (13.19%)
Koroleff Methods				
mean	**27.252**	**109.654**	**24.711**	**25.430**
repeatability	1.688 (6.19%)	1.022 (0.93%)	0.981 (3.97%)	0.742 (2.92%)
reproducibility	2.542 (9.33%)	7.769 (7.09%)	3.039 (12.30%)	3.610 (14.19%)
HTC Methods				
mean	**22.677**	**107.210**	**21.185**	**24.543**
repeatability	1.041 (4.59%)	2.649 (2.47%)	0.364 (1.72%)	0.518 (2.11%)
reproducibility	2.657 (11.72%)	10.73 (10.01%)	2.375 (11.21%)	3.304 (13.46%)

By variation of the preset number of injections, the preset limit of standard deviation and the allowed number of additional injections the 'accuracy' of the determination can be influenced in some range. As an increase in the maximum allowed number of injections also requires additional sample volume and time for analysis, each system is usually 'optimized' for the special requirements and conditions of the individual laboratory. This procedure of eliminating values with major contribution to standard deviation helps to minimize scattering of results, which may be caused by numerous external 'environmental' factors in the laboratory (e.g. electronical problems, influence of air conditioning systems, etc) and leads to highly reproducible values.
For the evaluation of the interlaboratory study it is assumed that reported results of HTC methods are all valid numbers from single injections of the sample (except those marked as invalid by the reporting laboratory itself). The mean values for the TN concentration in

the four samples calculated from the results of this group of laboratories are slightly lower than those calculated for all laboratories or those laboratories applying the Koroleff method (Tab. 1).
On the level of 5% probability value, the difference between Koroleff and HTC methods is significant for samples A and C. It must be noticed however, that – differing from those indicated in Annex Tab. 2 - some laboratories are included or excluded in this test on the basis of the separate evaluation of both groups according to DIN ISO 5725 (see Annex Fig. A1 & A2). The results are not necessarily the same if only those laboratories were excluded that are marked in Annex Tab. 2.

It was sometimes postulated in the past that those methods producing higher values for TN are the 'correct' methods, because higher values should be a proof for a more complete conversion of nitrogenous compounds into the detectable reaction product. However, in some within laboratory comparisons or in interlaboratory studies it was found that UV – methods sometimes produce even higher numbers than Koroleff method (Westernströer (1999), Sharp et al (2002b)). As on the other hand UV – methods had been suspected to perform 'incomplete' digestion, these results do not correspond with the above mentioned postulate and lead to a discussion about possible 'errors' in all methods which may cause overestimation of TN concentrations in some cases (Wangersky (2000), Sharp (2002d)).

There are some laboratories that report results always higher or lower than the mean calculated from the values of all participants. It should be checked by those laboratories whether there might be a problem with 'blank' or with the standard used for calibrating the analysis. As it is difficult to find these problems without appropriate reference material it is recommended to use reference material in every analysis. For DOC – measurements it is well documented that the use of reference material can improve the 'community precision' (Sharp (2002d)). The matrix of the reference material should be as close as possible to the samples which have to be analyzed. The use of reference material prepared in artificial media (e.g. in distilled water or artificial seawater) may sometimes cause problems in finding inconsistencies in the procedure applied (for example see Aminot & Kérouel (2001)).
Although some certified 'natural' reference material is offered from QUASIMEME, the availability of appropriate materials is limited or rather expensive and may therefore not be used in the frequency recommended. As a small number of the samples prepared for this interlaboratory study are still left they can be offered to other laboratories as 'reference material' to improve their TN analysis.

Participating laboratories were asked also to report results for other parameters than TN which were measured together with TN in the same analysis. A number of laboratories stated that they usually determine concentrations of other compounds together with TN, but they do not report the results. By reporting results in an interlaboratory study one

should keep in mind that additional data can help to prevent misleading interpretation of the results. As there is no absolute guarantee that all distributed samples are free of contaminations or errors during the filling and labeling process – even if every precautions were taken to prevent this – additional data are a great help to reveal those errors. Therefore it should be the concern of laboratories to provide as much information as available in interlaboratory studies in order to allow a well founded evaluation of the results.
In the TN interlaboratory study in summer 2002 only very few additional information was reported. Measurements of DP (dissolved phosphorous) were reported only by one laboratory. Concentrations of nutrients were available from three laboratories. As some laboratories applying the HTC method for the determination of TN measure TOC simultaneously, results for TOC were reported by four laboratories (Annex Tab. A5). Because of the low number of results, no detailed discussion should be given here. It should be mentioned however that laboratories 16 and 32 use (seawater) reference material to control the performance of the analysis and instrument blank.

As a result of this interlaboratory study it should be considered whether a detailed discussion of the results – including a discussion about details of the methods applied – should be started within the quality assurance system with all (or a group of selected) laboratories participating in this interlaboratory study. It is likely from the results that some improvements in the 'community precision' can be achieved, which would require only little effort in the laboratories.

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Annex Table A1 :

Alphabetical list of laboratories participating in the TN – interlaboratory study performed in summer 2002

1 Dorothea Altenhofen , Niedersächsischer Landesbetrieb für Wasserwirtschaft und Küstenschutz (NLWK) , Betriebsstelle Brake
2 Dr. Uwe Brockmann , Universität Hamburg , Institut für Biogeochemie und Meereschemie
3 Dr. Gustave Cauwet , Centre National de la Recherche Scientifique , Laboratoire Arago , Observatoire Océanologique , Banyuls-sur-Mer cedeex (France)
4 Dr. Horst Gaul , Bundesamt für Seeschiffahrt und Hydrographie , Hamburg
5 Dr. Michael Gluschke , Landesamt für Umwelt, Naturschutz und Geologie Mecklenburg-Vorpommern , Güstrow
6 K.-D. Hochfeld, Landesamt für Umwelt, Naturschutz und Geologie Mecklenburg-Vorpommern , Güstrow
7 Dr. Gerhard Kattner , Alfred-Wegener-Institut für Polar- und Meeresforschung , Bremerhaven
8 Fr. Adina Langenfeld , Niedersächsisches Landesamt für Ökologie , Hildesheim
9 Dr. Karin Lau , Niedersächsischer Landesbetrieb für Wasserwirtschaft und Küstenschutz (NLWK) , Betriebsstelle Cloppenburg
10 Helga Meyering , Niedersächsischer Landesbetrieb für Wasserwirtschaft und Küstenschutz (NLWK) , Betriebsstelle Meppen
11 L. Mollenhauer, Landesamt für Umwelt, Naturschutz und Geologie Mecklenburg-Vorpommern , Stralsund
12 Dr. Günther Nausch , Institut für Ostseeforschung , Warnemünde
13 Dr. Marianna Pastuszak , Sea Fisheries Institute , Department of Oceanograpy , Gdynia (Poland)
14 T. Petenati , Landesamt für Natur und Umwelt (LANU) Schleswig Holstein / Laborschiff MS 'HAITHABU'
15 Dr. Mireille Pujo-Pay , Centre National de la Recherche Scientifique , Laboratoire Arago , Observatoire Océanologique , Banyuls-sur-Mer cedeex (France)
16 Dr. Gerda Rünger , Landeslabor Schleswig-Holstein , Außenstelle Kiel II
17 U. Schleichert , Bundesanstalt für Gewässerkunde , Koblenz
18 Manfred Schulze , Niedersächsischer Landesbetrieb für Wasserwirtschaft und Küstenschutz (NLWK) , Lüneburg
19 Uwe Schweers , Niedersächsischer Landesbetrieb für Wasserwirtschaft und Küstenschutz (NLWK) , Verden
20 Dr. Irmgard Voigt , Niedersächsischer Landesbetrieb für Wasserwirtschaft und Küstenschutz (NLWK) Süd , Labor Braunschweig
21 Ulrich Wiegel , Niedersächsischer Landesbetrieb für Wasserwirtschaft und Küstenschutz (NLWK) , Betriebsstelle Stade
22 Ulrich Willers , Behörde für Umwelt und Gesundheit , Amt für Umweltschutz , Hamburg
23 Dr. Peter Woitke , Umweltbundesamt/FG II 3.6 , Labor für Wasseranalytik , Berlin
24 Dr. Elke Zwirnmann , Institut für Gewässerökologie u. Binnenfischerei , Berlin
25 Dr. Klaus Nagel, Institut für Ostseeforschung , Warnemünde

As some of the laboratories applied more than one method for the determination of TN – concentration, 29 data sets had been reported.

Annex Table A2 :

Mean values and s. dev. reported for TN in the interlaboratory study performed in summer 2002
All concentrations are in µM N

Analyte : TN (total nitrogen)								
Lab.No	Sample A		Sample B		Sample C		Sample D	
	mean	s.dev.	mean	s.dev.	mean	s.dev.	mean	s.dev.
1	***80.08***	1.11	128.50	1.27	***119.85***	0.35	26.59	0.80
2	25.26	1.51	111.38	6.03	22.40	0.35	25.43	0.37
3	19.30	0.11	96.54	1.74	**9.15**	0.09	15.33	4.15
4	26.04	1.01	109.50	2.12	24.63	0.55	21.72	0.39
5	**30.15**	2.49	111.94	0.95	22.65	0.55	25.30	0.11
6	26.35	0.13	116.84	0.67	23.51	0.09	24.80	0.03
7	- / -		136.00		- / -		23.60	
8	24.87	0.92	118.36	4.93	22.85	0.20	28.79	0.11
9	21.78	0.88	109.40	1.39	21.07	0.98	23.28	0.81
10	22.87	0.54	- / -		20.67	0.16	- / -	
11	***85.32***	0.50	- / -		***119.76***	3.28	27.88	1.97
12	***68.18***	0.51	122.45	5.59	***114.25***	2.05	30.70	0.00
13	**26.81**	2.72	106.15	6.64	22.19	1.75	23.75	1.70
14	28.50	1.51	109.00	2.00	28.90	1.61	**27.53**	3.00
15	25.47	0.35	101.22	2.45	23.47	0.32	21.98	0.62
16	25.78	0.69	125.80	2.06	23.40	0.66	28.38	0.61
17	23.76	0.00	100.06	0.58	21.79	0.16	23.42	0.00
18	25.22	0.01	122.25	7.00	25.15		24.52	0.15
20	21.93	0.42	115.00	0.98	27.93	1.65	23.79	0.76
21	28.83	1.16	**136.28**	9.65	26.84	0.14	30.81	0.60
22	30.70	0.99	105.35	0.49	30.70	0.00	32.15	1.06
23	**25.66**	2.47	98.55	0.19	22.37	1.48	21.77	1.08
24	25.46	0.34	111.10	0.82	23.54	0.47	25.40	0.01
26	18.87	1.28	103.63	3.05	18.20	0.22	23.59	0.37
27	**36.85**	7.64	114.75	0.45	**44.38**	2.44	**33.67**	5.42
28	22.90	0.00	95.00	3.26	21.23	0.67	30.35	0.40
30	24.72	0.24	107.16	5.39	22.87	0.61	24.89	1.21
31	23.01	1.26	**85.53**	14.61	20.36	1.29	22.63	0.84
32	24.30	0.28	97.90	1.84	21.85	0.35	24.15	0.64

Annex Table 2 : (continued)

Lab.No	Sample A		Sample B		Sample C		Sample D	
	mean	s.dev.	mean	s.dev.	mean	s.dev.	mean	s.dev.
mean	31.03		110.95		33.78		25.58	
S.D.	17.06		12.33		30.22		3.84	
%cv	54.96		11.12		89.44		15.01	
mean *)	25.42		110.95		23.68		25.58	
S.D. *)	3.73		12.33		5.86		3.84	
%cv *)	14.69		11.12		24.76		15.01	
mean **)	25.57		110.95		23.42		25.19	
S.D. **)	2.87		10.54		2.89		3.61	
%cv **)	11.67		9.50		12.35		14.50	

*) summary statistics calculated without the six values in bold and italics. Reasons for selecting these six numbers are discussed in the text (see #3.4)

**) summary statistics calculated without the data in bold or in bold and italics. Numbers in bold are rejected by the evaluation process according DIN ISO 5725 because of too large within laboratory variance (9 numbers) and/or too large systematic error in the level of test results (1 number).
if s.dev. is missing, only a single value is reported

Annex Table A3 :

Initial characterization of samples collected for the TN – interlaboratory study

	Sample A	Sample B	Sample C	Sample D
collection area	Baltic Sea eastern Gotland Basin (Stat. TF0271)	inshore lake Klein Kölzig (near Cottbus)	western Baltic Sea (Stat. TF0361)	sample prepared in laboratory
matrix	seawater (lower salinity)	lake water	seawater (higher salinity)	Creatinin in MQ - water
salinity	12.02		22.73	
concentrations [1])				
NO_2 / NO_3	0 [2])	6.23	7.41	
NH_4	20.18	131.4	1.20	
DN (UV – AAN)	[4])	162.88	[4])	
DN (HTC)	29.92		18.04	24.19 [3])
PN	1.99		2.81	
TN	31.91		20.05	24.19 [3])
DOC	283.2	[4])	162.8	32.25 [3])
POC	9.8	[4])	33.2	

[1]) concentrations in µmol / l N or C, respectively
[2]) sample taken from anoxic water layer
[3]) calculated from weighted pure substance
[4]) no data available

Annex Table A4 :

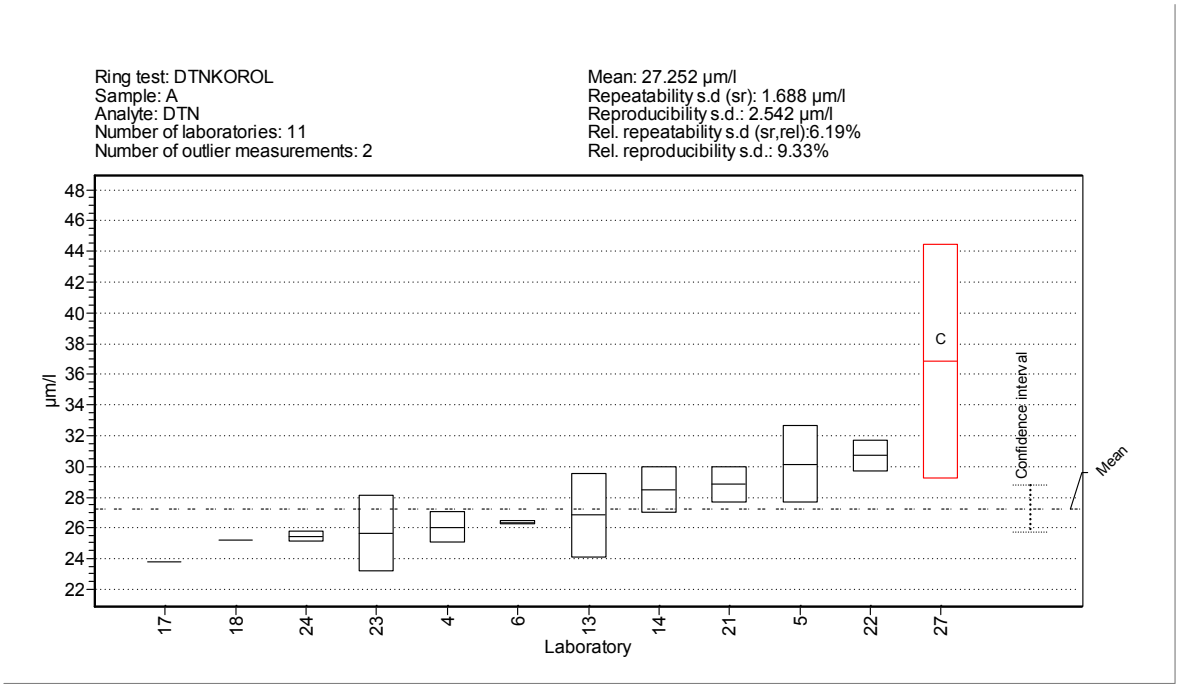
Concentrations in µM N (µM C for DOC) measured in the samples in spring 2002 shortly after filling the ampoules
Measurements are performed by the laboratory of the institute organizing the exercise

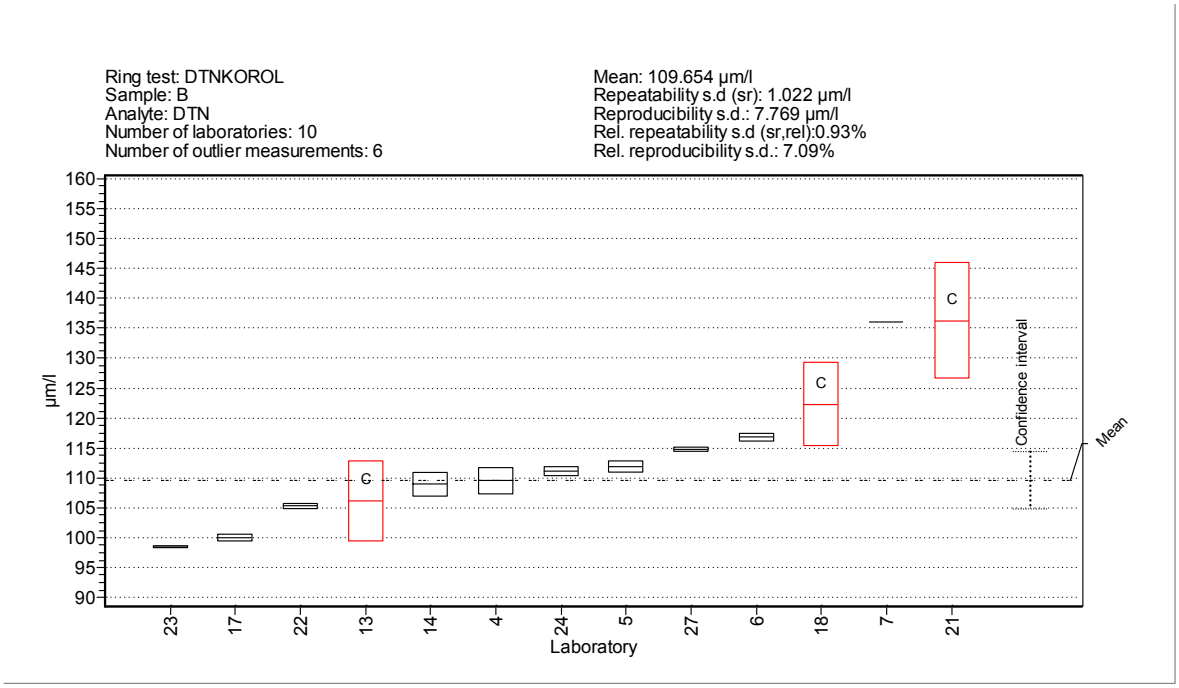
Parameter	Sample A	Sample B	Sample C	Sample D
NO_2 / NO_3 [1])	11.25 (± 0.12)	3.13 (± 0.26)	8.17 (± 0.13)	
NH_4 [1])	1.56 (± 0.46)	102.2 (± 2.00)	4.46 (± 0.55)	0.36 (± 0.08)
DN (UV – AAN) [2])	24.72 (± 0.24)	107.2 (± 5.39)	22.87 (± 0.61)	24.89 (± 1.21)
DN (HTC) [3])	23.01 (± 1.26)	85.53 (± 14.61)	20.36 (± 1.29)	22.63 (± 0.84)
DN (HTC – hybrid system) [4])	24.30 (± 0.28)	99.20 (± 1.84)	21.85 (± 0.35)	24.15 (± 0.64)
DOC	250.3 (± 10,95)	67.7 (± 4.90)	175.9 (± 8.85)	38.9 (V 4.70)

[1]) standard AutoAnalyzer protocol
[2]) online UV digestion / CFA (Skalar 'San-plus-System)
[3]) HTC – method performed on Skalar Formacs^{HT} TN – Analyzer / Skalar ND10 Detector
[4]) HTC – method performed on Shimadzu TOC5000 TOC- Analyzer / Skalar ND10 Detector

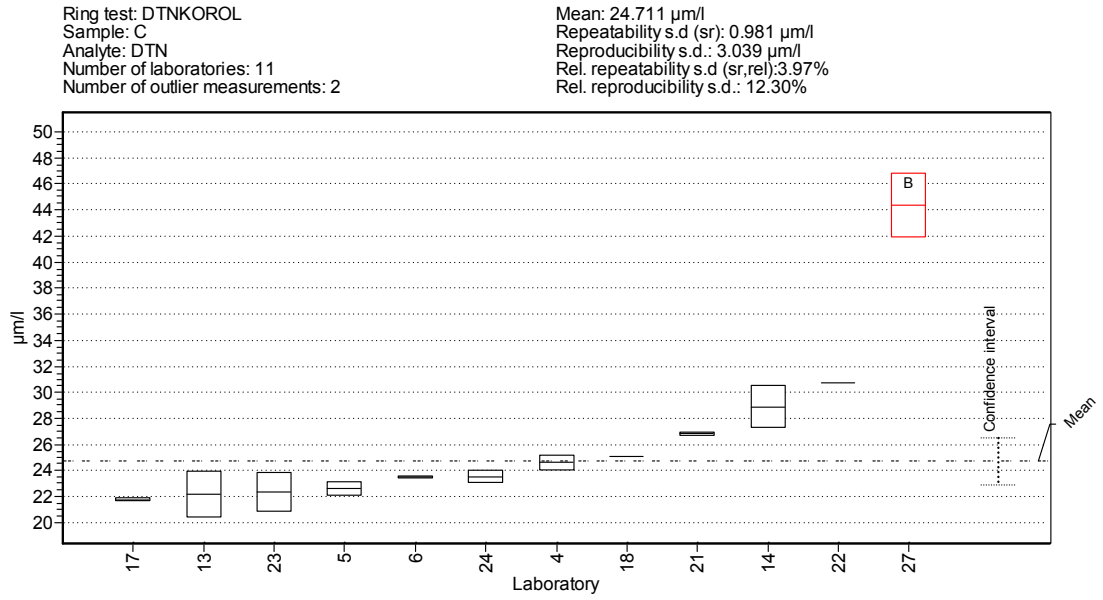
Annex Fig. A1 :

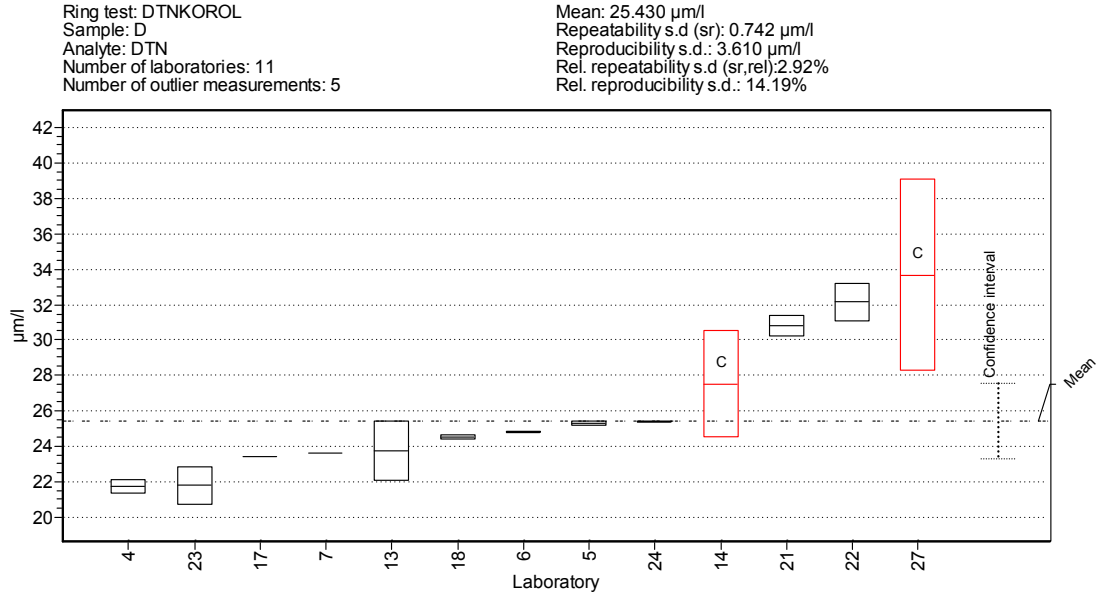
Evaluation of the TN – interlaboratory study in summer 2002 -
Evaluation based only on results obtained by wet digestion methods ('Koroleff – digestion') (but without Koroleff – digestion / direct UV determination of nitrate !)
Graphs are produced with the program PROLAB98 (Dr. Uhlig, quo data, Dresden) by Dr. P. Woitke and Dipl.-Ing. J. Wellmitz (FG II 3.5, Umweltbundesamt Berlin)




Ring test: DTNKOROL
Sample: B
Analyte: DTN
Number of laboratories: 10
Number of outlier measurements: 6
Mean: 109.654 µm/l
Repeatability s.d (sr): 1.022 µm/l
Reproducibility s.d.: 7.769 µm/l
Rel. repeatability s.d (sr,rel):0.93%
Rel. reproducibility s.d.: 7.09%
µm/l
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
C
Confidence interval
Mean
23
17
22
13
14
4
24
5
27
6
18
7
21
Laboratory

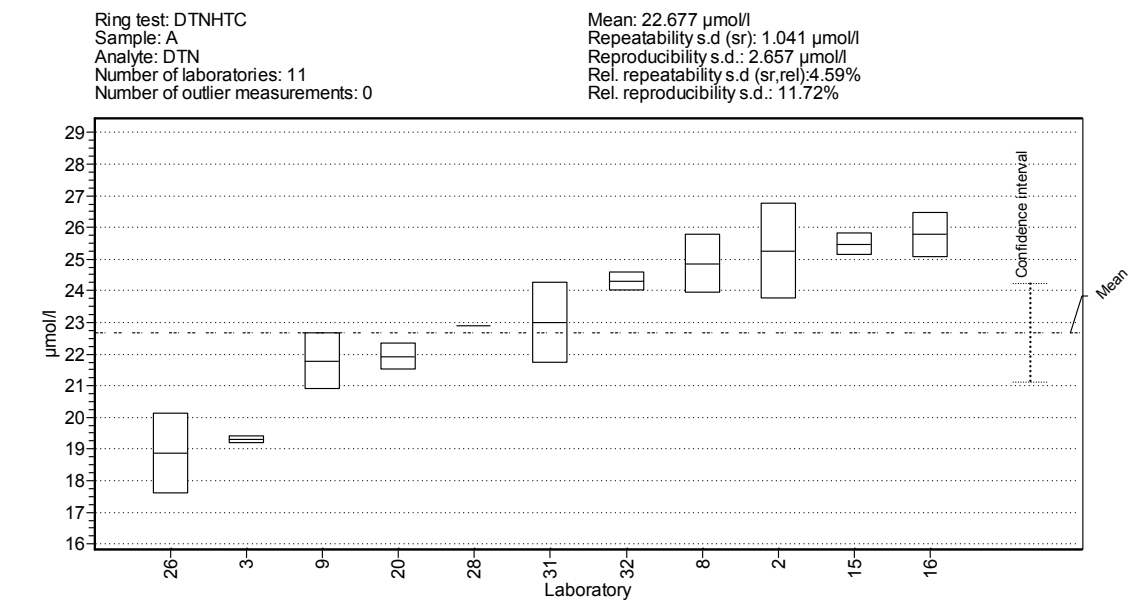
Annex Fig A1 : (continued)

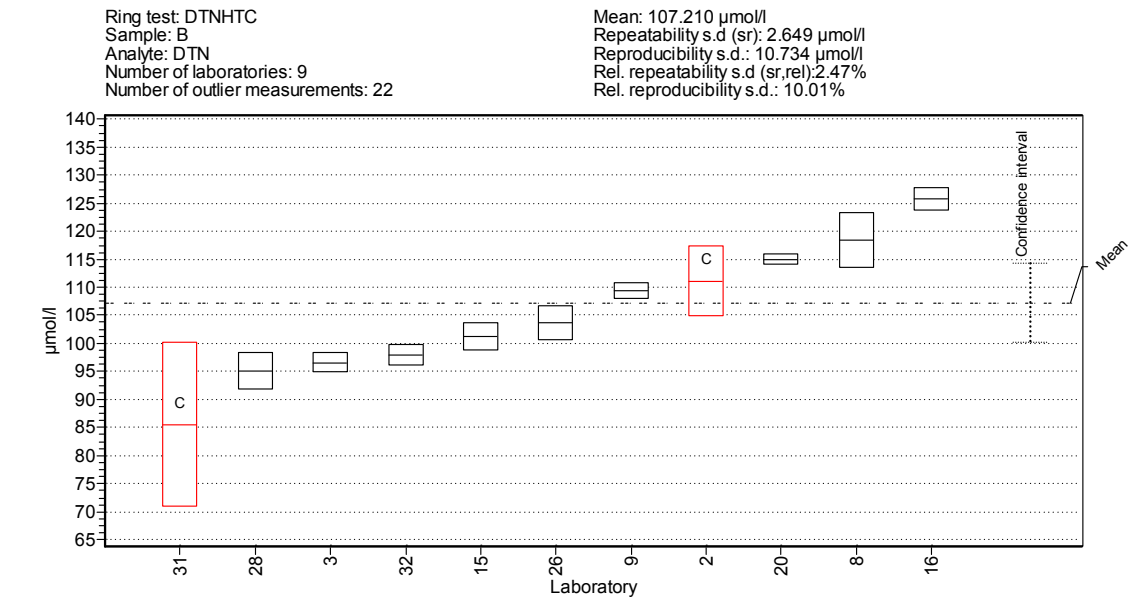




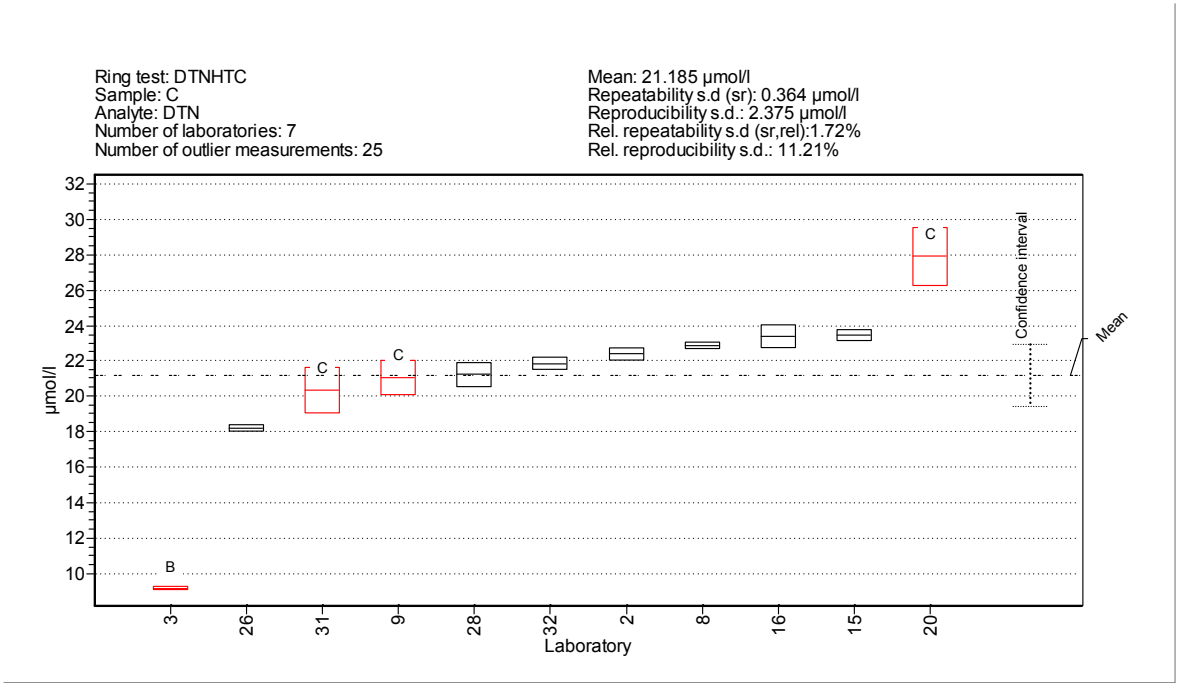
Annex Fig. A2 :

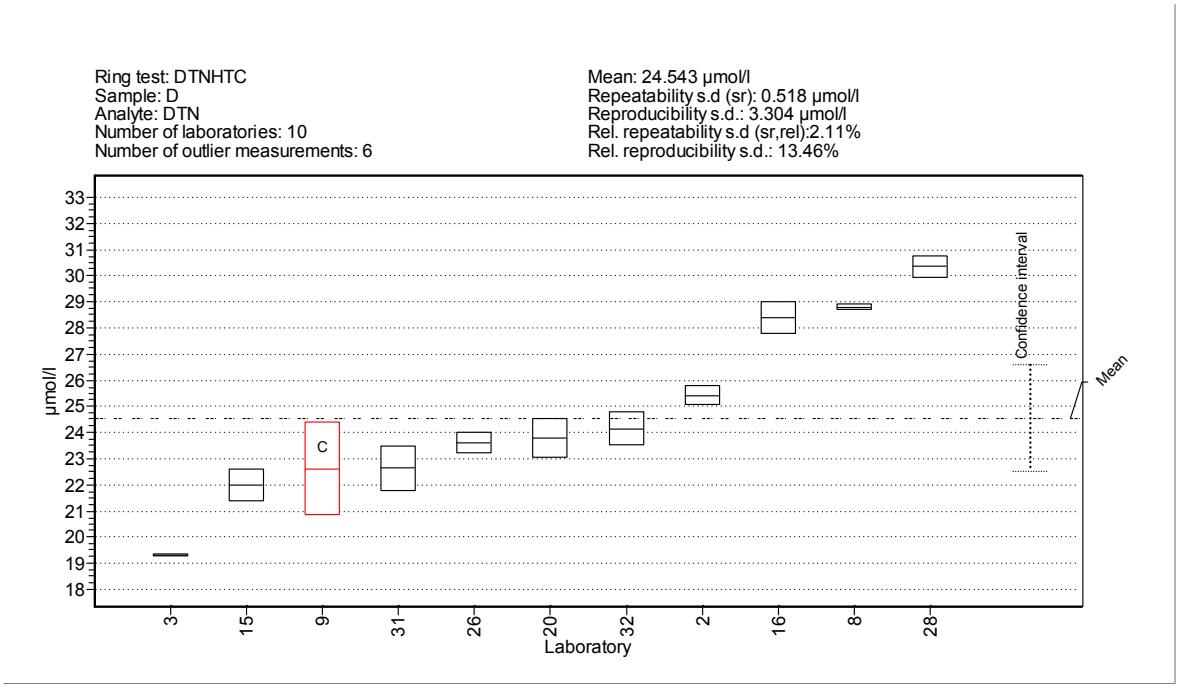
Evaluation of the TN – interlaboratory study in summer 2002
Evaluation based only on results obtained by the laboratories applying HTC methods !
Graphs are produced with the program PROLAB98 (Dr. Uhlig, quo data, Dresden) by Dr. P. Woitke and Dipl.-Ing. J. Wellmitz (FG II 3.5, Umweltbundesamt Berlin).





Annex Fig. A2 : (continued)





Annex Table A5 :

Mean values and s. dev. reported for TOC in the TN – interlaboratory study performed in summer 2002
All concentrations are in µM C

Analyte : TOC (total organic carbon)								
Lab.No	Sample A		Sample B		Sample C		Sample D	
	mean	s.dev.	mean	s.dev.	mean	s.dev.	mean	s.dev.
3	294.2	24.7	83.1	5.5	197.9	7.6	51.1	27.4
16	248.4	2.5	73.3	3.3	177.0	3.3	36.7	0.8
26	227.0	4.7	60.5	3.2	151.1	3.1	34.6	3.9
32	250.3	11.0	71.1	4.9	175.9	8.8	35.5	4.7