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TECHNICAL STUDY No. 15

IMPACT OF THE CATALYST (Cu or Se) ON THE ACCURACY OF MEASUREMENTS OF TOTAL KJELDAHL NITROGEN IN WASTEWATERS

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ABSTRACT

The determination of Total Kjeldahl Nitrogen (TKN) is a former method widely applied to many matrices. Se has replaced Cu as a catalyst in the past to reduce reaction time. In the environmental field, specifically in water, the current standard "NF EN 25663 (ISO 5663) 1994 Water quality - Determination of Kjeldahl nitrogen - Method after mineralization with selenium" will be reviewed in 2024. In this frame, given the toxicity of Se and current occupational health, safety, and environmental concerns, the question of replacing Se as a catalyst with Cu arises. Data from AGLAE's wastewater proficiency tests were used to assess the impact of this method's change over the measurement's accuracy. Switching from Se to Cu as a catalyst increases TKN results by 1% over the studied range of concentrations, i.e., between 35 and 90 mg N/L. In most cases, results obtained with Cu alone had a similar dispersion than those obtained with Se alone and occasionally slightly higher.
The statistical analysis shows that using Cu would only slightly affect the accuracy of determining Kjeldahl nitrogen in wastewater.

It seems conceivable that harmonisation (via standardisation) of analytical practices using Cu as a catalyst could reduce the observed dispersion of results. The conditions under which it is used (volume of test sample, mineralisation temperature/time, etc.) are heterogeneous.

CONTENT

1. INTRODUCTION

NF EN 25663 standard – “Determination of Kjeldahl nitrogen. Method after mineralisation with selenium” is equivalent to ISO 5663 standard. It specifies:
1/The transformation of nitrogen compounds that can be determined by the method, into ammonium sulfate by mineralisation with sulphuric acid containing a high potassium sulfate concentration (to raise the mixture's boiling point) with Se as a catalyst.
2/ liberation of the ammonia from the ammonium sulfate by adding a base, followed by distillation in a boric acid solution used as an indicator.
3/ ammonium ion determination in the distillate by titration with titrated acid (or direct determination of the ammonium in the mineralised sample by spectrometry at 665 nm).

The catalyst used in the past was Hg. It was then replaced by Cu and then by Se in the 1930s to reduce mineralisation time and, therefore, to increase the productivity of the method. In the environmental field, a wide variety of catalysts is used. These include reference mixes such as Devarda, Missouri, and Wieninger, whether produced industrially or by laboratories. However, there are also mixtures designed and validated by the laboratories based on Se, Cu, Hg, or Ti.

The next revision of NF EN 25663 standard is scheduled for 2024. Given the toxicity of selenium, it might be considered replacing it with another catalyst. Consequently, it is interesting to determine whether the results of the measurements to date have been conditioned by using one or other catalysts (Cu or Se).

ISO 5663: 1984 standard - "Water quality. Determination of Kjeldahl nitrogen. Method after selenium mineralisation" was adopted by AFNOR in 1994 as "NF EN 25663 Determination of Kjeldahl nitrogen - Method after selenium mineralisation". Institutions such as EPA, AWWA, APHA, and WEF have used a method with just Cu since 1993.

This study aims to establish the impact on the accuracy of TKN measurement results for wastewater if they were determined using only Cu as catalyst. It is intended to provide the regulatory authorities with information based on the experience gained from the interlaboratory tests organised by AGLAE.

2. MATERIALS AND METHODS

Materials

AGLAE has proposed Total Kjeldahl Nitrogen for years in four matrix types: sediments, reusable sludge, natural water, and wastewater. For the past five years, information about the type of catalyst used by each participating laboratory has been systematically requested. For solid matrices, the number of data is small and insufficient for statistical analysis. There is more data for natural waters, but the number is still too small, particularly for Cu, to make reliable statistical use. Finally, we carried out the study on wastewater, for which nearly 80% of the results were obtained with Se, followed by Cu (between 15% and 20% depending on the test).

The materials used for these tests were raw water from an urban wastewater treatment plant, sometimes doped or diluted. The range of covered TKN concentrations was from 35 to 90 mg N/L.

Table 1. Historical data of TKN levels in AGLAE tests since 2017 (all catalysts combined) and description of the water used (matrix) in the tests.

Test	TKN, mg of N/L	Matrix	Spiking
17M2B.2	41,20	**Inlet water to a WWTP**	Spiked with KH_2PO_4
18M2B.1	82,08	**Inlet water to a WWTP**	Spiked with $(NH_4)_2SO_4$
18M2B.2	46,75	**Inlet water to a WWTP**	Spiked with $K_4P_2O_7$ and $C_8H_6KO_4$
19M2B.1	54,53	**Inlet water to a WWTP**	Spiked with $K_4P_2O_7$
19M2B.2	57,71	**Inlet water to a WWTP**	No spiking
20M2B.1	87,63	**Inlet water to a WWTP**	Spiked with NH_4Cl
20M2B.2	38,71	**Inlet water to a WWTP (70%) diluted with tap water (30%)**	Spiked with NH_4Cl and KH_2PO_4
21M2B.1	64,38	**Inlet water to a WWTP (75%) diluted with tap water (25%)**	Spiked with NH_4Cl and KH_2PO_4
21M2B.2	36,91	**Inlet water to a WWTP**	No spiking
22M2B.1	41,45	**Inlet water to a WWTP**	No spiking
22M2B.2	65,66	**Inlet water to a WWTP**	Spiked with NH_4Cl

Conditions varied considerably from one test to the next:

- time (the study includes results reported over five years)
- participants (they were not necessarily the same from one test to the next)
- spiking (for TKN but also other parameters)
- spiking products (ammonium chloride or sulfate, etc.)

For 9 out of 11 tests included in this study, the matrix used was 100% WWTP inlet water (diluted with tap water for the two other tests).

Table 2. The minimum and maximum values for the characterization parameters are shown below

	pH, pH units	Conductivity, µS/cm at 25°C	Turbidity, NFU	total organic carbon (TOC) (mg of C/L)	Total suspended solids (TSS), mg/L	Chemical oxygen demand (COD), mg of O_2/L
Characterisation values for the matrix	7,51 - 8,11	900 - 1267	60 - 187	57 - 168	99 - 282	250 - 619

The tests on which the study is based included 130 participants on average (105 for Se and 25 for Cu). It should be noted that the water used in these tests has usually low nitrate/nitrite levels.

Given the role of this parameter in the characterisation and monitoring of quality, where TKN levels are generally the highest, it is logical to consider that this study covers most of the needs for TKN measurement results and that it might be extrapolated to natural waters.

Methods

In most interlaboratory tests organised by AGLAE, participants are asked to carry out repeated measurements on the materials sent. Truenness and precision estimates are therefore produced for each test. The table below shows the statistical test desing used to measure TKN (Table 3). Such a desing involves the analysis by each laboratory of a batch of samples consisting of two bottles, which are analysed under repeatability conditions by all participants. The repeated analysis of two bottles from the same batch by each participant makes it possible to estimate the heterogeneity of the samples' batch used in the test. Thus, batch heterogeneity does not spoil the inter-laboratory reproducibility values calculated during the tests.

Table 3. AGLAE's typical statistical design for TKN tests on wastewater.

Batch	Bottle label	Replicate
Batch X	Bottle 1	A11
		A22
	Bottle 2	B11
		B22

For this repeatability and reproducibility study of measurements, AGLAE's historical data issued by all available methods were used.

Results for TKN (Cu and Se catalysts combined) from wastewater tests conducted over the last five years (Programme 2B "INDICATORS IN WASTE WATERS") were grouped according to the catalyst used and then statistically reprocessed. For 11 tests, the newly assigned values (robust means "m") and the standard deviation of repeatability and reproducibility of these interlaboratory tests were obtained independently for results of participants who used only Se as the "TKN-Se" catalyst and only Cu as the "TKN-Cu" catalyst. These values are shown in the table below.

Table 4. Assigned values, standard deviations of repeatability and reproducibility for TKN from separate treatments according to the catalyst used.

Test	**TKN-Cu** **m,** mg of N/L	**number of data considered**	(mg of N/L)		**TKN-Se** **m,** mg of N/L	**number of data considered**	(mg of N/L)	
			s_r	s_R			s_r	s_R
17M2B.2	41,44	9	0,6617	1,5271	41,29	81	0,3871	0,9357
18M2B.1	84,41	14	0,6627	5,8410	82,00	82	0,6539	1,6545
18M2B.2	46,86	13	0,4117	0,8818	46,76	86	0,4708	1,0314
19M2B.1	54,89	14	0,6565	0,9347	54,48	89	0,4053	1,4723
19M2B.2	57,97	14	0,3800	1,0352	57,74	91	0,4888	1,4066
20M2B.1	88,18	12	0,9735	4,4834	87,36	90	0,7471	2,1092
20M2B.2	39,07	13	0,4482	1,0792	38,77	84	0,3685	0,8879
21M2B.1	65,21	16	0,7641	1,7584	64,19	93	0,5204	1,4625
21M2B.2	37,77	18	0,3537	2,2625	36,92	87	0,4514	1,1395
22M2B.1	41,37	17	0,3832	1,4682	41,45	87	0,3239	1,1643
22M2B.2	65,90	14	0,7003	2,5423	65,66	84	0,5352	1,4852

In about half of the cases, only the results from participants who started processing their samples up to two days after receiving them were considered for the statistical calculations to avoid overdispersion due to sample instability. For the other half of the cases, all the data were taken into account, and the treatment that led to a better estimate of the inter-laboratory error and the assigned value was always selected. All these values were calculated using an improved version of algorithm A of the ISO 13528 standard, as was done for the TKN results for all catalysts combined. Models for the variation of precision values depending on the content were also calculated from these data. The models used to establish these precision scales are those of ISO 5725 standard.

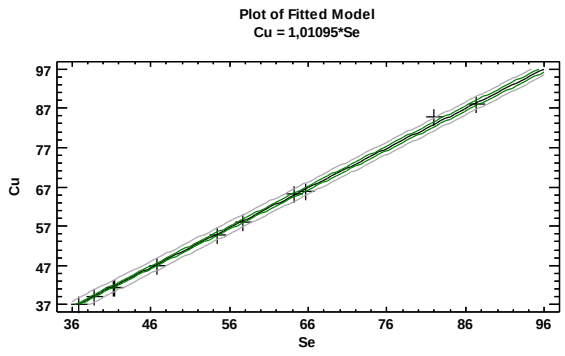
3. RESULTS & DISCUSSIONS

Trueness

A linear regression was carried out to identify a systematic bias between the measurements of the two methods (Cu-Se).
The equation initially found was Cu = -1.08445 + 1.02875*Se.

A Student's t-test to determine the significance of the coefficients showed that the intercept was not significantly different from zero. The regression was repeated to estimate a linear model with no intercept. The final model retained is Cu = 1.01095*Se.

Graph 1. Linear regression between TKN values obtained with Se and those obtained with Cu.



The confidence interval for the slope was calculated using the following formula:
« slope $\pm\, t_{(1-\alpha);p-1} \times {s_b}/{\sqrt{p}}$» in which :
Sb => standard error in slope estimation and t => Student's t for 95% and 99% confidence with the 11 data pairs studied (*p*).

Table 5. The proportionality factor and its confidence interval (at 5% and 1% confidence) estimated between Cu-Se results for TKN.

Low value at 1%	Low value at 5%	Slope	High value at 5%	High value at 1%
1,0000	1,0033	1,01010	1,0186	1,0219

That means there is a proportional bias with a factor equal to 1.01 between the results obtained with Cu and Se. In other words, the Cu method gives results 1.01% higher than those obtained with Se.
To summarise, in out of 11 tests on wastewater with levels between 30 and 100 mg N/L, we show that the results obtained with Cu were higher than those obtained with Se by a factor between 1 and 1.02. This bias is small but statistically significant.

Precision

From a precision point of view, switching from Se to Cu would not significantly deteriorate the dispersion observed.
To compare the dispersion of results obtained with Cu as the catalyst to that of results obtained with Se for each test, confidence intervals (CIs) on the repeatability and reproducibility of standard deviations (sr and SR) were estimated. The overlap of the CIs indicates the absence of significant differences between these standard deviations.

Table 6. Comparison of the confidence intervals at 1% and 5% risk of error for the standard deviations characterising the repeatability (sr) and reproducibility (sR) of the measurements per test for Cu and Se in the separate statistical treatments.

	17M2B.2		18M2B.1		18M2B.2	
	sr	sR	sr	sR	sr	sR
at 5%	Cu>Se	o	o	Cu>Se	o	o
at 1%	o	o	o	Cu>Se	o	o

	19M2B.1		19M2B.2		20M2B.1		20M2B.2	
	sr	sR	sr	sR	sr	sR	sr	sR
at 5%	Cu>Se	o	o	o	o	o	o	o
at 1%	o	o	o	o	o	o	o	o

	21M2B.1		21M2B.2		22M2B.1		22M2B.2	
	sr	sR	sr	sR	sr	sR	sr	sR
at 5%	Cu>Se	o	o	o	o	o	o	o
at 1%	o	o	o	o	o	o	o	o

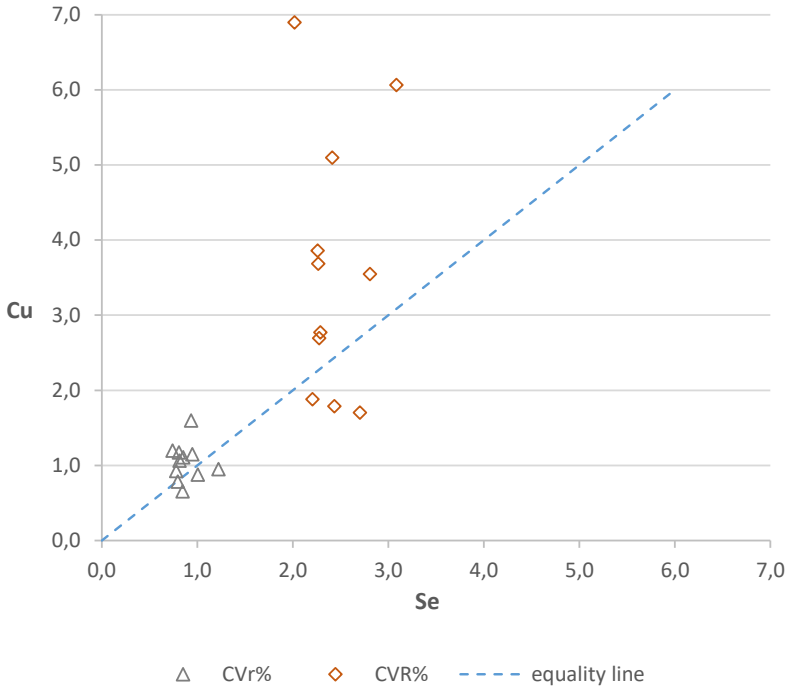
"o": standard deviations not significantly different
"Cu>Se": standard deviation with Cu as catalyst significantly higher than standard deviation with Se as catalyst

Repeatability and reproducibility standard deviations for measurements made with only Se and those with only Cu were similar in most cases. Specifically, on the comparison schema (11 tests x 2 precision levels x 2 confidence levels), significant differences were observed for only 4 cases:

- For 3 tests, the repeatability standard deviation of the measurements with Cu was found to be higher than that with Se with a risk of error of 5%.
- For 1 test, the repeatability standard deviation was higher for measurements made with Cu with a 1% risk of error.

These values were also compared using paired data tests to determine whether there was an overall tendency to have more significant standard deviations with Cu. These tests did not detect any significant difference between the standard deviations (sr and sR) obtained with Cu and Se. However, it can be seen that there are more tests with relatively higher standard deviations with Cu (see graph below).

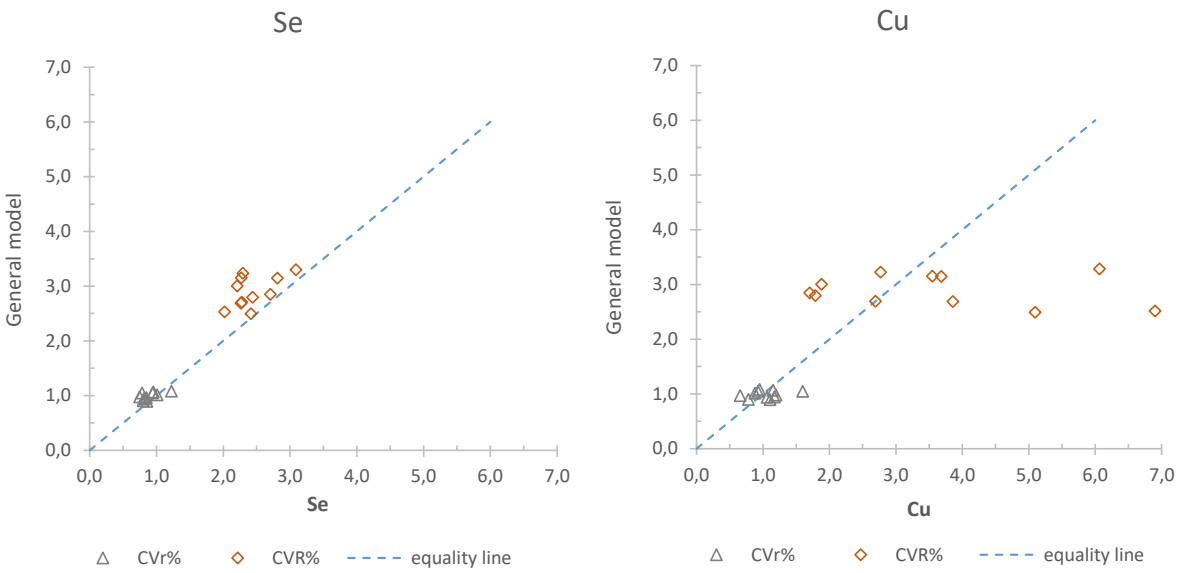
Graph 2. Repeatability (CVr%) and reproducibility (CVR%) values from separate Cu and Se treatments.



The ~~historical~~ dispersion chart for TKN measurements compiled by AGLAE contains the repeatability (CVr%) and reproducibility (CVR%) values observed since 1995. Based on these data, a general mathematical model has been calculated for more than 100 tests with levels varying between 5 and 100 mg N/L. Precision values from statistical treatment of the results obtained with just Se and just Cu were compared with those from the general model for TKN measurements (all catalysts taken together).

For Se, the values tend to be lower than those of the general model (mainly below the straight line of equality). This is quite logical, given that most of the data in the general model correspond to measurements taken with Se, but without the Cu values, which present a slight bias. For Cu, as observed for the 11 tests, the results dispersion may occasionally be greater than the dispersion of values obtained from the general model. Still, it remains similar in most cases (see graph below).

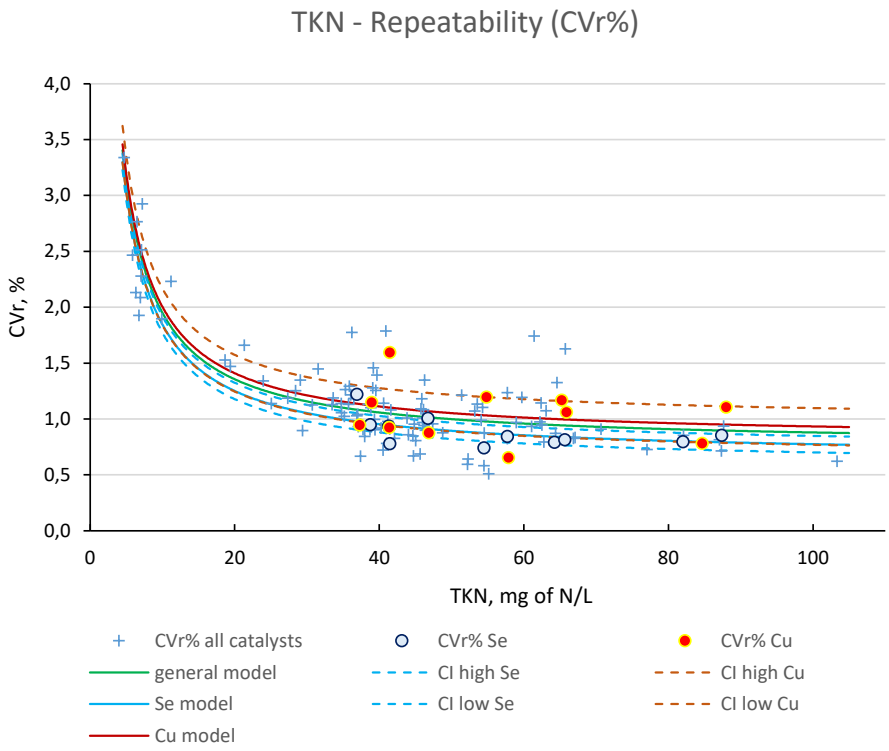
Graph 3. Repeatability (CVr%) and reproducibility (CVR%) values from the separate Cu and Se treatments compared with the values from the general model (all catalysts combined)

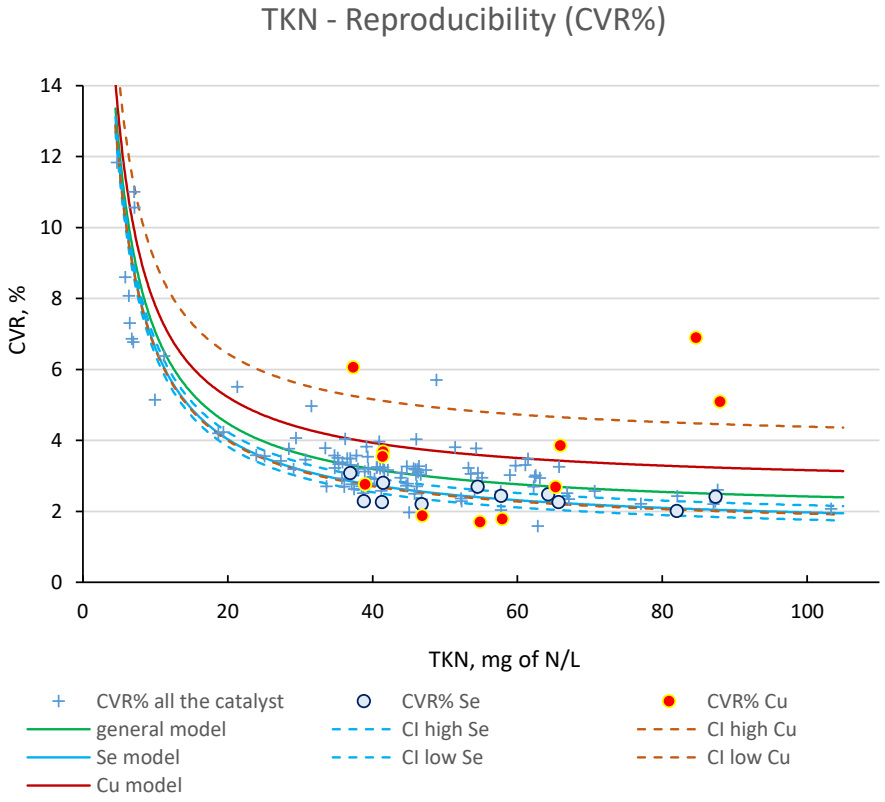


Nevertheless, it should be noted that the mean of the differences between the CVR% values obtained in the "all catalysts combined" tests and the values obtained from our separate treatments for Cu proved not to be significantly different from zero (likewise for the CVr% values). Thus, the lower and upper confidence limits for the mean of those differences were used to simulate a mathematical model that would characterise what would have been obtained if all the measurements had been made with just Cu as the catalyst (Cu_model). The model representing the results if the measurements had been made only with Se was also presented (Se_model).

These models are represented below, as well as 1/the patterns of variation of CVr% and CVR% as a function of the TKN content and 2/their 95% confidence intervals for all catalysts combined (general model) and for Se and Cu alone (Se_model and Cu_model, respectively).

Graph 4: Reproducibility and repeatability values for TKN for all catalysts combined (general model - green line), for results with just Se (Se model - blue line) and for results with just Cu (Cu model - orange line).





Repeatability values obtained with Cu and Se agree with the general repeatability model; all models and confidence intervals remain close to the CVr of 1% from 30 mg N/L.

As for reproducibility, for the "Se model", the confidence limits are close enough, which is logical given the large amount of data used for the estimation (80% - 90% of the data).

The "Se model" is reasonably close to the "general model" and falls below it (reproducibility is better). Most of participants use Se as a catalyst, so the data used to calculate the "Se" and "all catalysts combined" models are largely the same, but the fact that the results obtained for Cu are slightly biased implies that the dispersion of the data is smaller.

For the "Cu model", the confidence limits become wider because there are fewer measurement results per point for estimating the model. Nevertheless, we can see that the bounds encompass the "general model". Reproducibility, therefore, does not appear to be significantly different. However, we cannot exclude the risk that the reproducibility could be close to the upper limit of the confidence interval. Thus, rather than being over-optimistic, we will use as a reference point the estimates made for the case that would now be considered as the "worst case" scenario for the switch to Cu (upper limit of the Cu model), even though it is the model (solid line for Cu) that would represent the most likely situation.

Accuracy approach

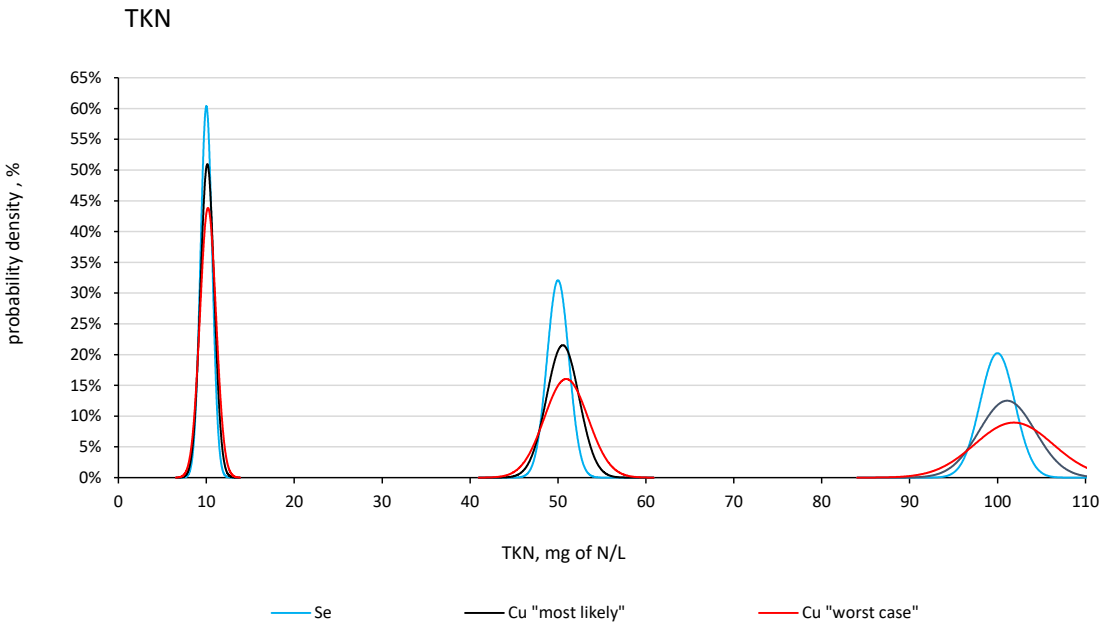
Several possible scenarios (for different TKN levels) are analysed in the graphs below, considering both the bias and reproducibility estimated above.
With the TKN concentration level on the x-axis and the probability density associated with the interlaboratory dispersion of the results observed for TKN on the y-axis, this graph shows the effect in terms of accuracy that could be observed when switching from Se to Cu.

A blue curve shows the distribution of expected values given the dispersion observed in an proficiency test with only Se ~~users~~ as catalysts for three materials covering the concentration range of interest with TKN contents of 10, 50 and 100 mg N/L. The black and red lines correspond to the results of a possible switch to Cu for the "most likely" case and for the "worst case", respectively.

These curves were plotted by calculating the normal distribution of the mean m equal to 10, 50 or 100 mg N/L, and the standard deviation "sR" found in the previous graphs for 10, 50 and 100 mg N/L. Two factors need to be noted when interpreting graph 5:

1/ Curves at 10 and 100 mg N/L are extrapolations. This study has no experimental points of comparison below 30 mg N/L or over 90 mg N/L.
2/ Proportionally, the dispersion is smaller at 100 than at 10 mg N/L. The dispersion at 100 mg N/L is, for example, ±5 mg N/L for Se, whereas it is ±2 mg N/L at 10 mg N/L. In other words, at 100 mg N/L it is ±5% and at 10 mg N/L it is ±20%.

Graph 5: Potential impact on TKN measurement results when only Cu is used as a catalyst.



According to this approach, if a material containing 50 mg of N/L of TKN (measured with Se as the exclusive catalyst - blue curve of graph 5, centered on 50) was analysed with Cu as the catalyst, this would give an average content of 50.55 mg N/L with 95% of the results lying between 46,84 and 54,25 mg N/L. If the material had a content of 100 mg N/L (measured with Se as the only catalyst), with Cu as the catalyst, the average content measured would be 101.10 mg N/L, with 95% of the results lying between 94,71 and 107,47mg N/L.

In addition, we can consider interpreting the consequences of this change of catalyst in terms of uncertainty. It was possible to estimate that the increase in the expanded relative uncertainty (U) caused by the switch to Cu would be, in the worst case, almost 5% bias included. This degradation can be considered slight, practically equal to half of the most frequently uncertainty value reported by the laboratories during our tests (11%).

Furthermore, the additional interlaboratory error could be reduced by harmonising analytical practices. A survey among participants of the 2B programme revealed that the analytical procedures of laboratories using Cu are quite different.

Some laboratories state that they follow NF EN 25663: 1993 standard by changing the catalyst (substitution of the Se catalyst by a Cu catalyst, without Se) and by adapting other points of the standard; others follow the NF EN 25663: 1993 standard by changing only the catalyst (substitution of the Se catalyst by a Cu catalyst, without Se) but by meeting the rest of the requirements of the standard. Others have developed an in-house analytical method.

In all three cases, the analytical conditions described are quite different:

- Test sample volumes of 100, 250 and 400 ml
- Mineralisation temperatures 345°C, 520°C and ramps from 290 to 330°C
- Mineralisation times: 2h, 150 min - 185 min, 215 min
- Salt/acid ratio for the mineralisation reagent (in g of Cu salt / mL of concentrated H_2SO_4 acid) of: ¼; ¾
- Setting up checks with nicotinic acid to determine digestion efficiency: some do, some du not—those who do not use acetamide or L-Glutamic acid, for example.
- Use of NH_4Cl to monitor nitrogen losses: some do, some do not.

Harmonisation of these analytical practices using Cu as a catalyst would undoubtedly reduce the interlaboratory error we observed during our tests. Finally, switching to this catalyst, which is less polluting for the environment, would provide a measurement uncertainty comparable to the current one.

4. CONCLUSIONS

Data from proficiency tests on wastewaters for matrices that are quite diverse in terms of time, nature, and spiking show that the TKN values estimated by laboratories using Cu as a catalyst (without Se) are only 1% higher than the results of laboratories using Se. A potentially wider dispersion of results could accompany this small but significant bias when using Cu. However, the additional measurement uncertainty would remain negligible compared with the median uncertainty currently reported by laboratories for this analysis.

It should be noted that this study does not deal with the analytical aspects (change in reaction time and temperature, among others) necessarily linked to the shift of catalyst, which must also be taken into account for a more complete approach of the subject, bearing in mind that the initial reason for switching from Cu to Se was productivity (faster analysis).