

1 **Multi-year interlaboratory exercises for the analysis of illicit drugs**
2 **and metabolites in wastewater: development of a quality control**
3 **system**

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Highlights

First worldwide inter-laboratory exercise for analysis of illicit drugs in wastewater

Results revealed (pre-)analytical issues for certain analytes

Six years of exercises have resulted in optimized procedures and protocols

Quality control system will make wastewater-based epidemiology results more reliable

79 **Abstract**

80 This study presents the development of a worldwide inter-laboratory testing scheme for the analysis
81 of seven illicit drug residues in different matrices (standard solutions, tap- and wastewater). By
82 repeating this exercise for six years with participation of 37 laboratories from 25 countries, the
83 testing scheme was substantially improved based on experiences gained across the years (e.g. matrix
84 type, sample conditions, spiking levels). From the exercises, (pre-)analytical issues (e.g. pH
85 adjustment, filtration) were revealed for some analytes which resulted in formulation of best-
86 practice protocols, both for inter-laboratory setup and analytical procedures. The results illustrate
87 the effectiveness of the inter-laboratory testing scheme in assessing laboratory performance in the
88 framework of illicit drug analysis in wastewater. The exercise proved that measurements of
89 laboratories were of high quality (> 80% satisfactory results for 6 out of 7 analytes) and that
90 analytical follow-up is important to assist laboratories in improving robustness of wastewater-based
91 epidemiology results.

92

93 **Keywords**

94 Illicit drugs; wastewater; inter-laboratory testing; wastewater-based epidemiology; quality assurance

95 **1. Introduction**

96 The measurement of the human excretion products of illicit drugs in influent wastewater has been
97 recognized as an alternative and complementary approach for estimating the consumption of illicit
98 drugs within communities, i.e. the catchment of wastewater treatment plants (WWTPs) [1-3]. The
99 principle behind wastewater-based epidemiology (WBE) derives from the fact that parent
100 compounds and/or their human metabolites (i.e., drug residues) are excreted in urine and faeces
101 following illicit drug use and end up in urban sewer systems [3]. The ability of WBE to provide useful
102 and timely information on temporal (daily, weekly, monthly, and annually) and spatial (within- and
103 between-countries) variations in illicit drug consumption has been demonstrated [4-15]. The
104 European Monitoring Centre for Drug and Drug Addiction (EMCDDA) has recently acknowledged the
105 added value of WBE to socio-epidemiological methods, such as population surveys, seizure data and
106 crime statistics, in generating useful and relevant data on population drug use [3].

107
108 With the aim to improve and optimize WBE, a Europe-wide collaboration was initiated in 2010. Seven
109 European institutions – University of Antwerp (BE), Eawag (CH), University Jaume I (ES), Mario Negri
110 Institute (IT), KWR Watercycle Research Institute (NL), Norwegian Institute for Water Research NIVA
111 (NO), and University of Bath (UK) - established the research group SCORE (Sewage analysis CORE
112 group Europe) [16]. The ultimate goals of SCORE are (a) to collaborate in the field of WBE to provide
113 reproducible data; (b) to improve and harmonize the analytical procedures used in different
114 laboratories to analyze drug residues in wastewater samples; and (c) to perform international studies
115 comparing illicit drug consumption in communities across the world. To this end, SCORE has
116 coordinated monitoring studies and exercises to assure the quality of reported data based on agreed
117 best-practices tackling sampling, storage and analysis. Important results from this collaboration are
118 multi-city studies demonstrating the usefulness of WBE on an international level to obtain the most
119 recent data on illicit drug consumption [17-18].

120
121 In order to further optimize and fine-tune WBE, it is imperative to gain knowledge on the sources of
122 uncertainties that are associated with the approach. In 2013, SCORE performed a thorough
123 evaluation on the uncertainties of WBE using the best-practice protocols and data that were
124 available from the comparative Europe-wide WBE research [19]. One of the cornerstones of WBE is
125 to accurately quantify concentrations of drug residues in wastewater samples by means of reliable
126 analytical procedures [20]. This requires fully validated analytical procedures before routine analysis
127 can be initiated and participation in external quality control schemes is, where possible, highly
128 recommended. External quality control through inter-laboratory exercises are based on the

129 distribution of the same test samples (in our case prepared by NIVA) to all participants. The latter
130 analyse all test samples without any knowledge of the concentrations of target analytes and return
131 their results to the coordinator of the exercise (in our case Eawag, who does not analyse test samples
132 and does not know the nominal spike value until final compilation of results). The coordinator
133 converts the submitted results into objective scores that reflect the performance of individual
134 laboratories and the group. These scores can alert participants of unexpected problems and can
135 result in actions to be taken [21].

136
137 SCORE initiated inter-laboratory exercises in 2011 in order to develop a quality control scheme for
138 laboratories that analyze illicit drug residues in wastewater for WBE purposes. Since its debut, the
139 testing scheme has been carried out annually with increasing participation of different laboratories,
140 also extending the network outside Europe. The objectives of the presented interlaboratory exercise
141 are (a) to illustrate the results of the six-year inter-laboratory testing scheme; (b) to evaluate
142 advancements achieved over these years and to identify issues still to be resolved; (c) to formulate
143 recommendations for future inter-laboratory exercises and (d) to propose a robust quality control
144 system to improve the analytical performance of laboratories analyzing illicit drugs in wastewater.

145 146 **2. Setup of the inter-laboratory exercises**

147 *2.1. Target analytes*

148 A total of seven illicit drug residues were targeted in the inter-laboratory testing scheme. These
149 included cocaine (COC), benzoylecgonine (BE, cocaine metabolite), 3,4-methylenedioxy-
150 methamphetamine (MDMA), amphetamine (AMP), methamphetamine (METH), 11-nor-9-carboxy-
151 tetrahydrocannabinol (THC-COOH, THC metabolite), and 6-monoacetylmorphine (6-MAM, heroin
152 metabolite). These analytes are widely regarded as the main urinary biomarkers of the worldwide
153 most consumed illicit drugs (COC, MDMA, AMP, METH, cannabis and heroin) and are the focus of
154 most bioanalytical and WBE initiatives around the world [22]. Certified spiking solutions of each of
155 the target analytes were supplied by Cerilliant Corporation (Round Rock, Texas, USA). All spiking
156 solutions were supplied in sealed glass ampoules at 1 mg/mL in methanol.

157 158 *2.2. Design of the exercises*

159 The basis of the inter-laboratory testing scheme was to compare the performance of the analytical
160 procedures employed by participating laboratories. Two separate modules were included to evaluate
161 in each laboratory (a) the use of correct analytical reference standards and the performance of the

162 instrumental analysis (Module 1), and (b) the performance of entire analytical procedures applied to
163 the analysis of wastewater, including sample preparation (Module 2).

164
165 For Module 1, a methanol solution containing the seven target analytes was used. For Module 2,
166 samples of tap water and wastewater spiked with the seven analytes were employed. Participants
167 were asked to use their own in-house developed and validated analytical procedures for the analysis
168 of the samples. Replicate analysis of each sample was requested ($n = 5$ for Module 1 and $n = 3$ for
169 Module 2). Commonly, sample pre-treatment consisted of filtration followed by solid-phase
170 extraction for Module 2 samples. All laboratories employed liquid chromatography coupled to mass
171 spectrometry using mass-labelled internal standards to perform detection and quantification of the
172 analytes. More information on different techniques, including sample preparation procedures, used
173 for this type of analyses can be found in Castiglioni et al. (2013) and Hernandez et al. (in press) [19-
174 20].

175 Analyte stability in various matrices and conditions is a crucial aspect of any inter-laboratory exercise
176 as it can substantially affect the outcomes of the analyses, particularly in the absence of certified
177 reference material in target matrices. Stability of illicit drugs in wastewater has been the subject of
178 numerous investigations, which were recently reviewed by McCall et al. (2016) [23]. Detailing the
179 results from all these studies goes beyond the scope of the present paper, however, a brief overview
180 regarding the analytes targeted in this inter-laboratory exercise is reported here. Both COC and BE
181 have been shown to be stable in wastewater over multiple weeks when stored refrigerated ($4\text{ }^{\circ}\text{C}$ and,
182 ideally, $-20\text{ }^{\circ}\text{C}$), at low pH and in the dark. Similarly, MDMA, AMP and METH have been shown to be
183 stable under similar conditions. THC-COOH and 6-MAM, on the other hand, have been shown to be
184 very sensitive to temperature and, for THC-COOH, low pH.

185 186 *2.3. Preparation of test samples*

187 All test samples were prepared by the Norwegian Institute for Water Research (NIVA). Figure 1 and
188 Table 1 give an overview of the type of test samples included in each year (2011-2016) and the
189 nominal spiking levels used. The two modules together comprised three matrices (i.e., methanol, tap
190 water and wastewater) spiked at different concentrations for each of the target analytes. Spiking
191 concentrations for all matrices changed from year to year to avoid bias and ensure legitimate results.
192 Certified spiking solutions (1 mg/mL in methanol) were diluted to prepare working solutions at $100\text{ }\mu\text{g/mL}$
193 or $10\text{ }\mu\text{g/mL}$ in methanol. The working solutions were then used to prepare different test
194 samples.

195 The methanol solution (Module 1) containing the analytes was prepared from each of the $100\text{ }\mu\text{g/mL}$
196 working solutions. Aliquots (1 mL) of this methanol sample were then transferred to separate glass

197 vials and capped. Each vial was accurately weighed and stored at -20 °C ahead of shipment to the
198 participants. Participants were asked to weigh the samples at arrival and to report deviations from
199 the weight at preparation.

200 Spiked wastewater and tap water samples (Module 2) were prepared in a 20 L high-density
201 polyethylene (HDPE) plastic container pre-washed with tap water and methanol. Twenty litres of cold
202 tap water or fresh wastewater from VEAS WWTP in Oslo (Norway) were poured into the container,
203 spiked with different volumes of the 10 µg/mL working standard solutions to obtain relevant
204 concentrations (at ng/L range) and stirred for 2 h to homogenize the mixture. In 2012, one of the
205 wastewater samples was used as it is; no spiking with target analytes occurred.

206 Samples from Module 2 were acidified to adjust the pH to 3.5 in 2012 and 2013. This pH adjustment
207 was agreed upon by the organizers of the exercise as at that time it was assumed that acidification of
208 samples was the best way to prevent degradation of the analytes [19]. In 2014-2016, no pH
209 adjustment of the tap water was performed because of the new insight into the negative effect of
210 low pH on the stability of THC-COOH in wastewater [23-24]. The changes in used matrices and pH
211 conditions across the years of the inter-laboratory exercise were the result of experiences of
212 previous years and of advancements made in the field of WBE.

213 Aliquots of at least 250 mL were placed in HDPE containers and stored at -20 °C before shipping to
214 the participants. As real wastewater was used, and which likely contained unknown concentrations
215 of the target analytes, it was not possible to use a genuine “blank” wastewater sample and nominal
216 values could thus not be reported. Instead, a total value, comprising background concentrations (x)
217 and the spiked level, was computed (Table 1).

218

219 *2.4. Participants and sample shipping*

220 The inter-laboratory exercises were organized by SCORE and were open to interested participants
221 from any institution. In order to participate to the exercise, laboratories were required to register
222 (without any payment) following an invitation sent out by SCORE or through the SCORE website [16].

223 Over the period between 2011 and 2016, a total of 37 laboratories from 25 countries participated in
224 the exercises (for more details on participation in each year, see Table 1). Most of the participating
225 laboratories (81%) were located in Europe, while the rest (19%) was spread over different continents
226 (North-America, Asia and Oceania) (Figure 2). The participants located within the European Union
227 received the test samples, shipped on ice, during the following 24-48 hours while for the remaining
228 participants from the other continents the average transport time was 2-4 days. Temperature during
229 shipment was not recorded, but participants were asked to not analyse samples if defrosted upon
230 reception (responsibility if the participant).

231

232 *2.5. Evaluation of results*

233 Participating laboratories were required to report measured concentrations of the target analytes in
234 each sample type provided. Results of individual replicates were submitted. Furthermore,
235 participants had to clearly highlight when concentrations were not quantifiable (i.e., below limits of
236 quantification) or when the analysis for a certain compound was not performed. Limits of
237 quantification for each participant were estimated with a fixed protocol and compared to self-
238 assessed limit of quantifications. It was established at a signal-to-noise ratio of 10 using the
239 quantifier transition from chromatograms of samples spiked at the lowest validation level tested. The
240 estimated limits of quantification were for all participating laboratories within the same order of
241 magnitude and comparable to what was reported by each lab based on validation data. Since 2015,
242 one spiking level was used to evaluate whether the analytical procedures of participants had limit of
243 quantifications that are relevant in the context of WBE studies. If participants could not report values
244 for this sample, they were notified that their analytical procedures did not reach relevant sensitivity.
245 First, the mean concentration (m) of replicates for each participant and for each sample type was
246 calculated. Secondly, after testing for normality, a Grubbs' test was performed to identify outliers
247 which were excluded from further analysis. From the remaining means, the group's mean [i.e., mean
248 of means (M)] and the group's standard deviation (SD) were computed. To evaluate the performance
249 of each participant (i), z-scores (z_i) for every analyte and sample type were calculated as follows:

250
$$z_i = \frac{m_i - M}{SD}$$

251 Following the ISO standard, a laboratory passed the inter-laboratory exercise when its $|z| \leq 2$ [21,
252 25]. Participants with results that were identified as outliers (Grubb's test) or had $|z|$ -values > 2 were
253 individually notified about the deviation and were allowed to recheck their submitted values for
254 inconsistencies or errors. Note that no detail (z_i , M) was supplied with the notification of the
255 deviation in order to maintain impartiality. If these laboratories were able to supply a viable
256 explanation (such as transcription errors), they were allowed to resubmit corrected results. If
257 accepted, newly submitted values were used to compute updated values for m_i , M , SD and z_i .
258 The purpose of this iterative process lies in the goal of SCORE to advance and improve WBE. The
259 inter-laboratory exercise was therefore used to assist laboratories in optimizing their analytical
260 procedures and improve the overall performance.

261

262 **3. Results and Discussion**

263 *3.1. Assigned value: group's mean vs. nominal concentration*

264

265 The z-score was calculated relative to the group's mean (M). The main reasons for using M instead of
266 the nominal concentration (i.e. spiking levels) as reference in the context of this inter-laboratory
267 exercise are [21, 25]:

- 268 (i) Multiple scientific evaluations repeatedly revealed that spiking concentration levels did
269 not necessarily display sufficient reliability to be used as an assigned value to calculate z-
270 scores;
- 271 (ii) For wastewater samples, the use of spiking levels as assigned value is out of the question
272 because of the presence of unknown concentrations of the analytes (no nominal values
273 exist);
- 274 (iii) There is a sufficient number of laboratories that participated in the exercises along the
275 years (Table 1);
- 276 (iv) Certified reference materials (CRMs) for analyzing illicit drugs in water samples are not
277 available;
- 278 (v) No recognised reference laboratories for this type of analysis exist;
- 279 (vi) The chosen approach was agreed by the participants as they were all informed on the
280 calculation and evaluation procedures applied.

281
282 Figure 3 shows the deviation of the group's mean (M) from the nominal concentration (spiking level)
283 for the methanol and tap water test samples. For the wastewater samples included in the exercises
284 from 2012-2014, it is impossible to generate any meaningful plot because of the unknown
285 background concentrations of the analytes present in this matrix.

286 The results showed that the deviation of the group's mean (M) from the nominal concentration was
287 mostly < 25%, which was regarded by SCORE as an acceptable variability. The deviation for the
288 matrix-free samples (i.e., methanol solvent) was mostly well below this 25% limit and suggested that
289 in all laboratories, the reference standards (both native and isotope-labelled) used and the
290 instrumental analysis (e.g. calibration and instrumental parameters) did not lead to substantial bias
291 in the analysis of the target analytes, except for 6-MAM. However, in the presence of matrix,
292 deviations of more than 25% occurred more often, in particular for 6-MAM and THC-COOH.
293 Concentrations of 6-MAM were systematically underreported, for both the standard solution and tap
294 water samples. In some occasions, the deviation amounted up to 60%. This systematic
295 underestimation of 6-MAM could be due to: (i) inaccuracies during the preparation and spiking of the
296 test samples (e.g. preparation and dilution of stock solution); (ii) stability issues of this analyte during
297 preparation of the test samples and during storage and sample handling; (iii) issues with the
298 analytical procedures applied by the laboratories.

299 The analysis of THC-COOH in the methanol samples gave acceptable results (deviation <25% and no
300 systematic error), while deviations of up to 90% were observed in tap water samples in 2013 and
301 2014. It is important to highlight that tap water samples were acidified in 2013 and, in the following
302 year, sample acidification before filtration was still performed by multiple participants. These were
303 later shown to have a negative impact on the measured concentrations of THC-COOH because of
304 adsorption issues [23-24, 26]. Acidification may be the cause of the high variability observed for this
305 analyte, but this is clearly not the whole picture. In fact, Causanilles et al. (2017) demonstrated that
306 different (combinations of) parameters (pH, filtration, sorption) can have an influence on the analysis
307 of THC-COOH in wastewater [26].

308 For COC, all samples across the different years showed deviations <25%, except for the three tap
309 water samples in 2015. The nature of this systematic deviation (only one year) indicates the error
310 likely occurred in the preparation of these test samples.

311

312 *3.2. Influence of different matrices and concentration levels on the group's variability*

313 The influence of the different matrix types on the performance of participating laboratories was
314 assessed through analysis of the datasets from all years. Figures 4 and 5 illustrate the influence of the
315 three matrices on the relative standard deviation (RSD) of the group. Overall, a lower RSD for the
316 methanol samples compared to the waste- and tap water samples was observed (Wilcoxon rank sum
317 test p -value $< \alpha = 0.05$). This observation was not surprising considering that concentrations of the
318 standard solution samples were in the $\mu\text{g/L}$ range while in tap water and wastewater, samples
319 concentrations were in the ng/L range. Furthermore, analysis of the methanol solution samples did
320 not require any substantial sample preparation (i.e., direct injection with/without further dilution)
321 compared to waste- and tap water samples, which required pre-concentration. A significant
322 difference between the RSDs for tap water and wastewater samples was observed (Wilcox rank sum
323 test p -value = 0.01, $\alpha = 0.05$). For THC-COOH, high RSDs were observed for tap water and wastewater
324 samples compared to the other analytes. Likewise, in the methanol solution, high RSDs were
325 observed on several occasions (Figure 4). These findings further suggest that there are some issues
326 with the analysis of this particular compound in water samples, as discussed earlier (Figure 3).

327 The difference in RSDs between tap and wastewater samples was further investigated using ANOVA
328 (after log transforming the data to correct for deviation from normality and heteroscedasticity).
329 Statistical analysis revealed that the spiking level showed the most significant influence on the
330 group's RSD ($F(1,98) = 121.5$, $p < 0.0001$), followed by the matrix type ($F(1,98) = 10.9$, $p < 0.001$) and
331 the compound under analysis ($F(6,98) = 3.0$, $p < 0.01$). Because the matrix type was not the most
332 influential parameter, the use of spiked tap water samples was deemed adequate for the purposes of
333 the present inter-laboratory exercise. In fact, when using wastewater samples, (a) differences in

334 matrix effects occur between locations and (b) background concentrations of the analytes in
335 wastewater are unknown and uncontrollable. As a result, it was not considered possible to use
336 'representative' wastewater for the purpose of this inter-laboratory exercise. Furthermore, by using
337 tap water, labour and logistic costs linked to the preparation and distribution of additional samples
338 to the participants could be reduced significantly. Issues related to the biodegradation and sorption
339 of target analytes in wastewater during shipment could also be reduced. Furthermore, our study,
340 including data over a six-year period, provides unique insights into how the molecular properties of
341 the analytes, concentration levels and matrix type affect laboratory performance in the context of
342 (waste)water analysis. The information and experience gained could hence be useful for other inter-
343 laboratory exercises confronted with similar matrices.

344

345 *3.3. Performance of laboratories*

346 The evaluation of the results obtained by all laboratories discussed hereafter is based on the
347 performances with the spiked tap water samples, as this matrix was shown to be appropriate (see
348 section 3.2) and because of the issues with wastewater samples mentioned earlier (i.e., unknown
349 background concentrations and potential stability issues). Figure 6 provides an overview of the
350 proportion of satisfactory results per analyte type in the period of 2013-2016. A satisfactory result is
351 regarded as a $|z|$ -value ≤ 2 [21, 25]. Grubb's outliers, non-detects (reported as below limit of
352 quantification) and $|z|$ -values > 2 are regarded as unsatisfactory. In the supporting information,
353 detailed results for each laboratory over the different years are shown. The plots give an overview of
354 the distribution of the z-scores of the group for the different years, matrices and spiking levels and
355 detailed plots for results of the individual laboratories (including intra-laboratory variation).

356 In general, for BE, COC, MDMA, and AMP, the group's performances were acceptable, with $> 90\%$ of
357 satisfactory results. For METH and 6-MAM, the satisfactory result were around 80% in 2013. This can
358 be linked to the fact that 3 out of 15 (METH) and 3 out of 10 (6-MAM) participants did not detect the
359 analytes in the test samples. In 2014-2016, acceptable results for these two analytes were obtained,
360 probably due to the higher concentration levels and improved performance of the analytical
361 procedures of the participants. The unsatisfactory results obtained for THC-COOH analysis over years
362 have drawn the attention of SCORE and triggered a further investigation of the effect that different
363 pre-analytical steps (filtration and pH adjustment) have on the accuracy the analysis of this
364 compound in wastewater [26].

365 It is important to mention that the aim of SCORE is to improve the reliability of WBE studies.
366 Therefore, support was provided to laboratories that showed unsatisfactory results by means of
367 short-term visits of a SCORE member and/or optimization of the analytical procedures (assistance
368 with sample preparation and method validation). In most cases, this resulted in positive outcomes

369 for these laboratories in following exercises. This highlighted the need for follow-up of inter-
370 laboratory exercises combined with a continuous support to all participants.

371

372 The z-scores regarding different concentrations of each analyte were visualised in scatter biplots (i.e.,
373 Youden plots, Figure 7) to assess the sources of variability among the participating laboratories.
374 Inter-laboratory variation predominates if results were clustered in the upper right and lower left (=
375 white) quadrants, while intra-laboratory variation predominates if results are clustered in the upper
376 left and lower right (= grey) quadrants [25]. Furthermore, the distances of the plotted point relative
377 to the 45-degree reference line and to the (0, 0) point (i.e. the Manhattan median) are both useful
378 for the interpretation of inter-laboratory data. Points that lie close to the 45-degree reference line
379 but far from the Manhattan median indicate a systematic error. Points that lie far from the reference
380 line suggest large random errors. The majority of the participating laboratories was found within the
381 white quadrants (Figure 7), meaning that inter-laboratory variability was predominant over the intra-
382 laboratory variability for all seven analytes. Only a few laboratories were occasionally outside of the
383 $|z|$ -values > 2 boundaries. For the latter, this implies large total errors, which were mainly
384 systematic, as results were close to the 45-degree reference line but distant from the origin.
385 Moreover, it should be noted that no recurrent erroneous results were observed, i.e., there were no
386 laboratories with anomalous results for a certain analyte reported across different years. This
387 supports the hypothesis that the observed errors were rather incidental and/or that these
388 laboratories had improved their analytical procedures.

389

390 *3.4. Sources of variations and recommendations*

391 The six-year data from inter-laboratory exercises for the analysis of illicit drug residues in water
392 samples revealed variations linked to its setup and allowed to provide recommendations to improve
393 future exercises. First, this study shows that the group's mean should be used to evaluate
394 performance of laboratories rather than the nominal (spiked) value. However, it is important that
395 nominal values should always be considered to exclude pre-analytical issues, as demonstrated for
396 THC-COOH. This observation triggered further investigations and recommendations to improve the
397 WBE approach to estimate cannabis use [26]. Second, since concentration levels were found to be
398 the main factor influencing performances (Figure 4, see section 3.2), spiking levels should be chosen
399 carefully, and reflecting concentrations expected in real samples. Particularly, for the methanol
400 standard samples, the use of different concentrations (e.g. Youden couple) instead of a single (high)
401 level, as we did, will be useful to improve the assessment of laboratory performances. Third, it is
402 important to prepare and transport test samples in the most optimal way in order to avoid stability
403 and adsorption problems. The issues observed with 6-MAM and THC-COOH when samples were

404 acidified (see section 3.1) are a good example and highlight the need to consider other preservatives
405 (e.g., sodium metabisulphite ($\text{Na}_2\text{S}_2\text{O}_5$) or sodium azide (NaN_3)) to ensure analyte stability during
406 transport and storage [27-28]. Furthermore, future inter-laboratory exercises should include an extra
407 analysis of the test samples by the preparing laboratory directly after preparation of the test samples
408 before freezing and shipment. This will improve understanding of the differences between the
409 nominal spike and the assigned value.

410 Based on the experiences acquired from these six rounds of inter-laboratory exercises,
411 recommendations related to analytical procedures used by individual laboratories for measuring
412 illicit drugs and metabolites in wastewater can be formulated. Laboratories can freely choose their
413 preferred sample preparation procedure and detection/quantification technique, but we strongly
414 suggest that the methods comply with the following features. First, mass-labeled internal standards
415 should be used for each analyte and spiked in samples before any filtration step. Second, pH
416 adjustment - when needed - has to be conducted after internal standard spiking and/or filtration.
417 This is particularly relevant for the analysis of THC-COOH in wastewater [26]. Third, freeze-thaw
418 cycles of the samples should be minimized. Fourth, in-house quality control samples (e.g. spiked tap
419 water or wastewater) should be prepared and analysed with each sample batch. Furthermore,
420 centrifugation instead of filtration can be an alternative way to avoid the blockage and clogging of
421 solid-phase extraction cartridges with particulates present in the wastewater.

422

423 **4. Conclusions**

424 This study presents, for the first time, the results of an inter-laboratory testing scheme for the
425 analysis of illicit drugs and metabolites in wastewater. By repeating this exercise for six years, we
426 were able to improve the set-up of the testing scheme substantially, based on experiences gained
427 over the years (e.g. matrix to be used, sample parameters, spiking levels) and to establish a reliable
428 quality control system. The existence of such system is important to ensure high-quality data of WBE
429 monitoring studies that can be used by stakeholders to obtain the most recent data on spatial and
430 geographical trends in illicit drug use on a national and international scale.

431 The results of the exercise highlighted the importance of using the group's mean rather than the
432 nominal value as the assigned value, in particular due to the lack of certified reference materials for
433 testing illicit drugs in wastewater. An investigation of the RSD associated with reported results
434 showed that the most influential parameter was the spiking level, not the instrument (method) used
435 or the type of matrix (i.e., tap or wastewater). Consequently, tap water was chosen for future
436 exercises as it presents various advantages. Specifically, it allows to control spiking levels more easily,
437 which is not possible with wastewater as unknown background concentrations exist. In fact,

438 substantial variations in composition and analyte concentrations occur, even within wastewater
439 collected from a unique location.

440 Regarding laboratories performances, the results from the inter-laboratory exercise show that these
441 were generally satisfactory for COC, BE, MDMA, AMP and METH. An improvement was observed
442 over the years and, in its latest round in 2016, more than 90% of the participating laboratories
443 reported results $|z|$ -value ≤ 2 . In the case of 6-MAM and THC-COOH, results from the exercise
444 showed that important pre-analytical issues still exist, and that sample pH has an important influence
445 on the stability of the latter analytes. Whilst these issues still need to be solved, it is important to
446 notice that none of the participating laboratories repeatedly (i.e., systematically) reported erroneous
447 results for the same analyte across multiple years, emphasising the improvements in analytical
448 performances which took place over the years.

449 The results illustrate the effectiveness of the inter-laboratory testing scheme in assessing and
450 improving laboratory performance in the framework of illicit drug analysis in wastewater. The
451 exercise proved that measurements of individual laboratories were of high quality and that analytical
452 follow-up is important in order to assist laboratories in improving the robustness and accuracy of
453 WBE results. The set-up and procedures used in this exercise for the measurement of illicit drugs in
454 wastewater and experiences gained during the six-year period are of importance for the
455 development of other quality control systems dealing with the measurement of pharmaceuticals,
456 personal care products and other contaminants in aqueous matrices.

457 Wastewater-based epidemiology has gained importance, as numerous national and international
458 organisations rely on its measurements to improve quantification of illicit drug use. Consequently,
459 additional efforts will be needed in future to ensure the impeccable quality of reported results and
460 tackle the existing and upcoming challenges. In particular, improving analytical performances for
461 important compounds such as 6-MAM and THC-COOH and, at the same time, adapting protocols to
462 integrate an ever growing number of relevant substances (e.g., new psychoactive substances) are
463 among the main challenges that laboratories will face in future.

464

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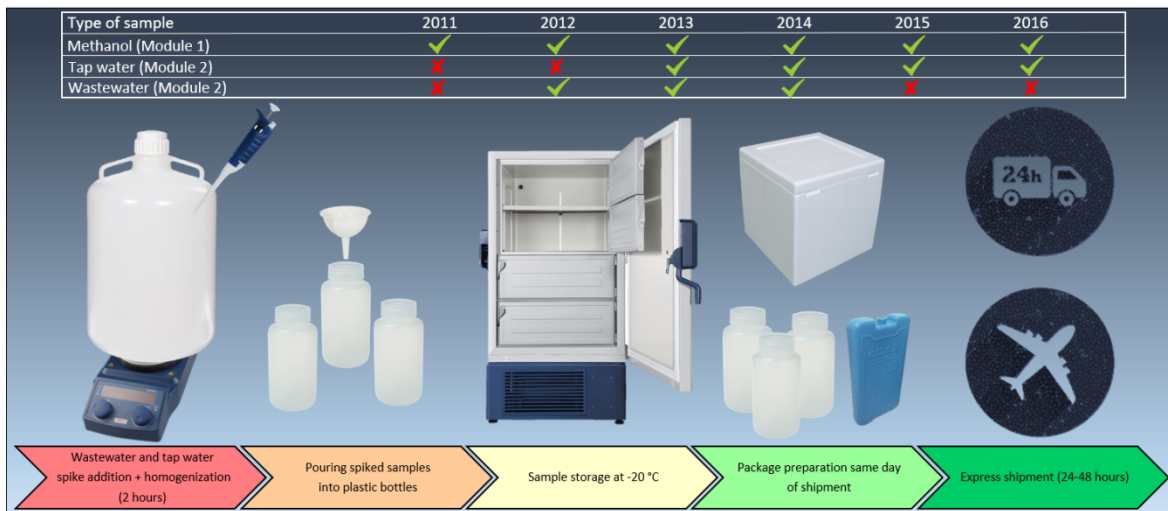


Figure 1. Inter-laboratory overview and scheme of the sample preparation and shipment for Module 2.

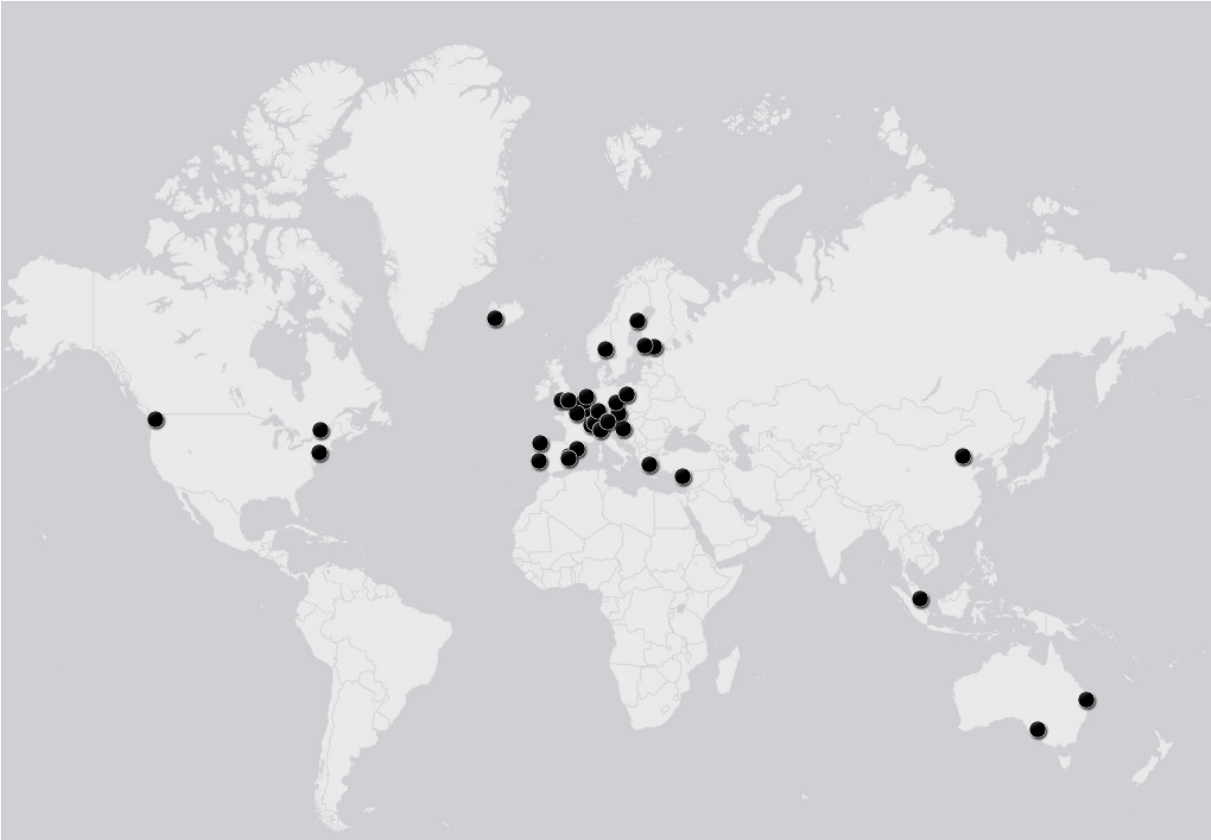


Figure 2. Map with location of the participants of the inter-laboratory exercises

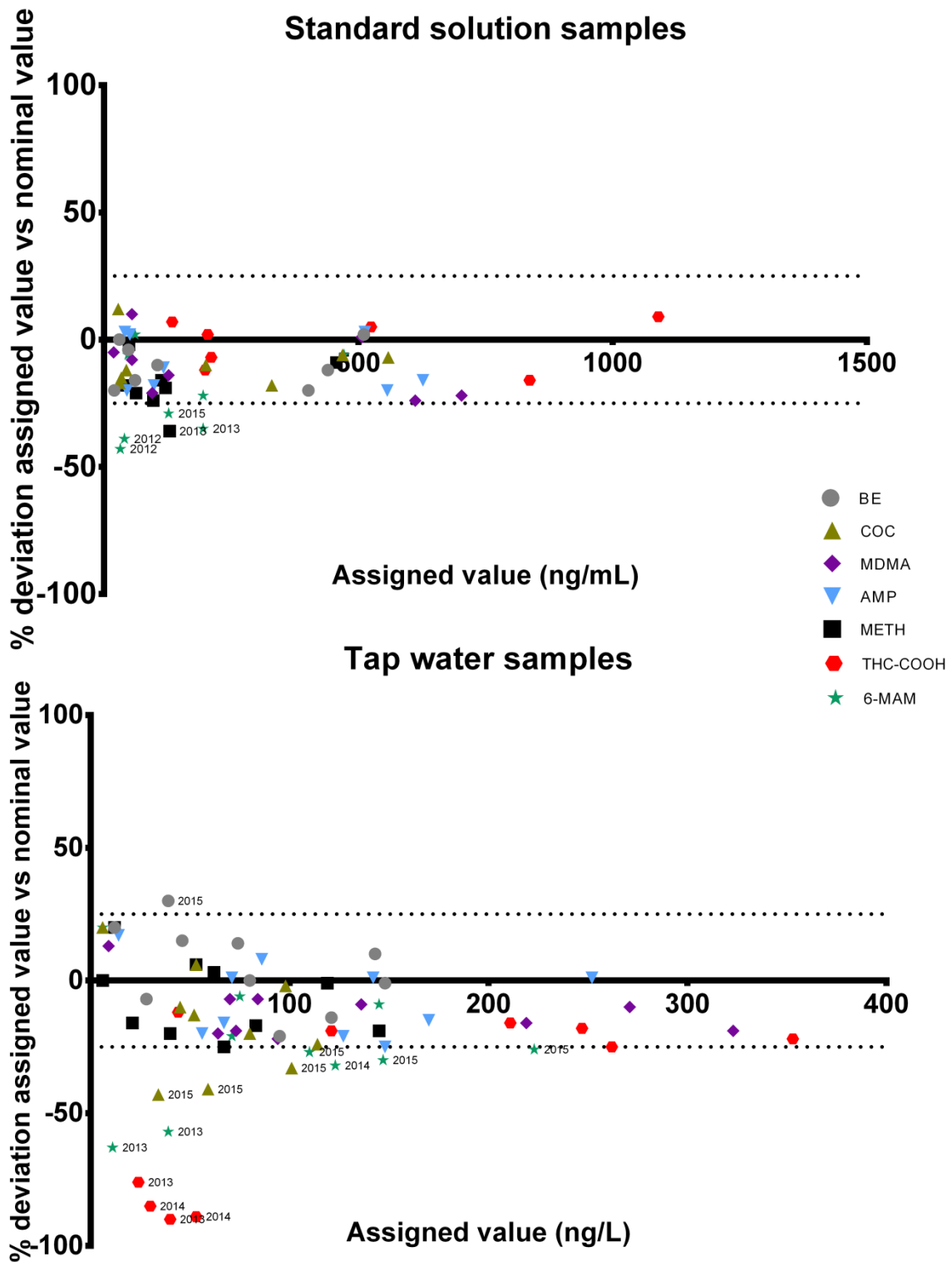


Figure 3. Deviation of the assigned value (= group's mean) from the nominal value (= spiking level) for the standard solution (top) and the tap water samples (bottom) in relation to the assigned value for the seven analytes. The dotted line represents 25% deviation. Entries with deviations > 25% are marked with the year of the inter-laboratory exercise.

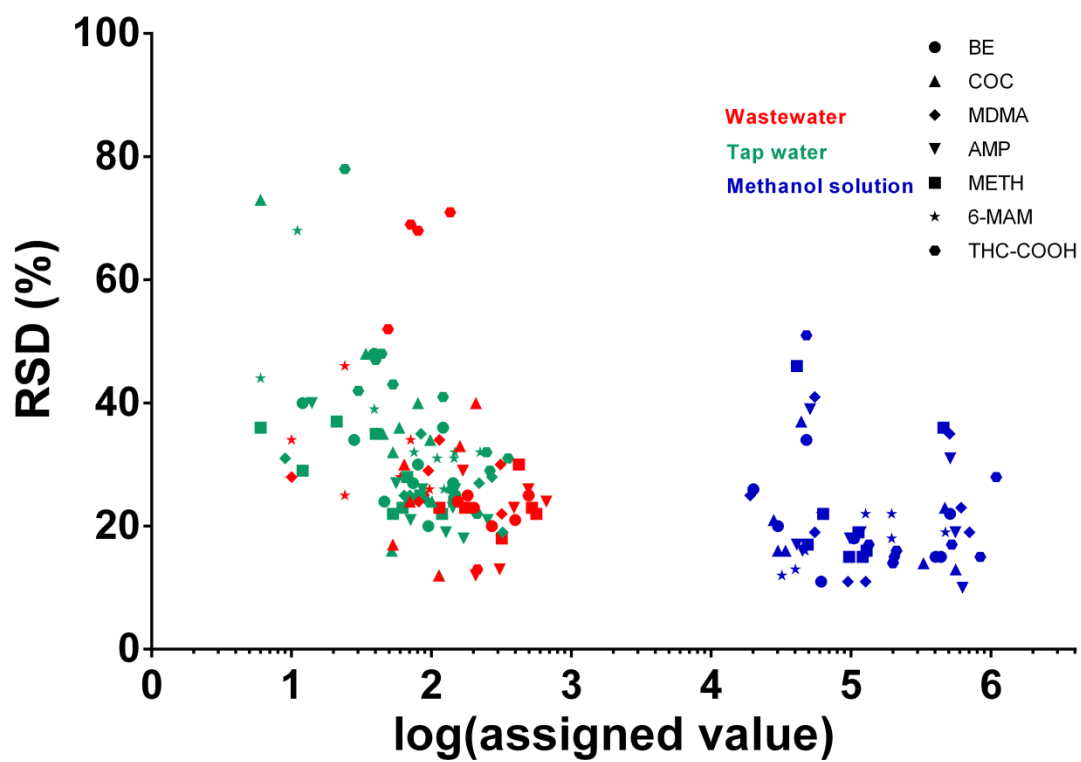


Figure 4. Relative standard deviation of the group in relation to the assigned value M (logarithmic scale) for the three matrices [standard solution (blue), tap water (green) and wastewater (red)] and seven analytes. All years (2011-2016) included.

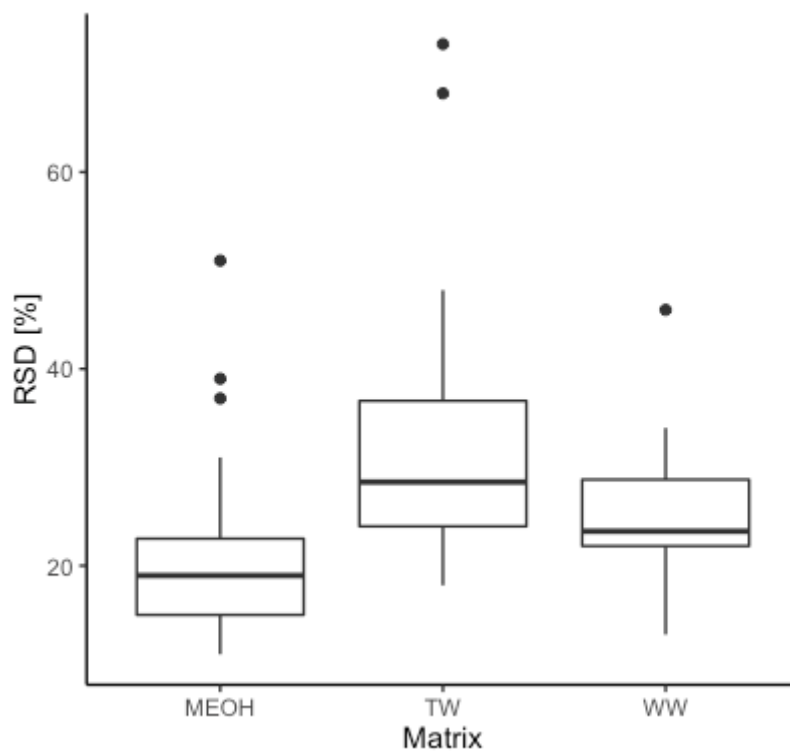


Figure 5. Boxplot showing the difference in the group's RSD for the three different matrices (MEOH = standard solution; TW = tap water; WW = wastewater) in 2013 and 2014 for all analytes.

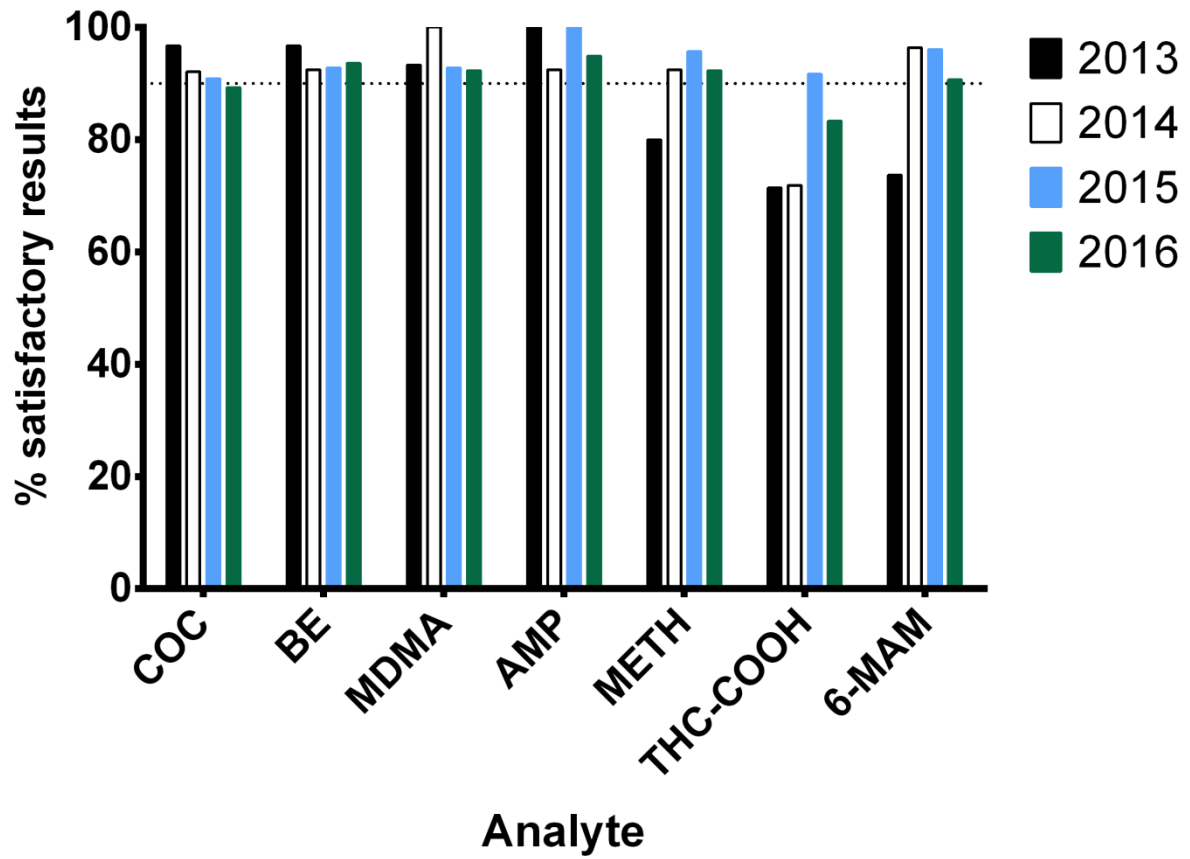
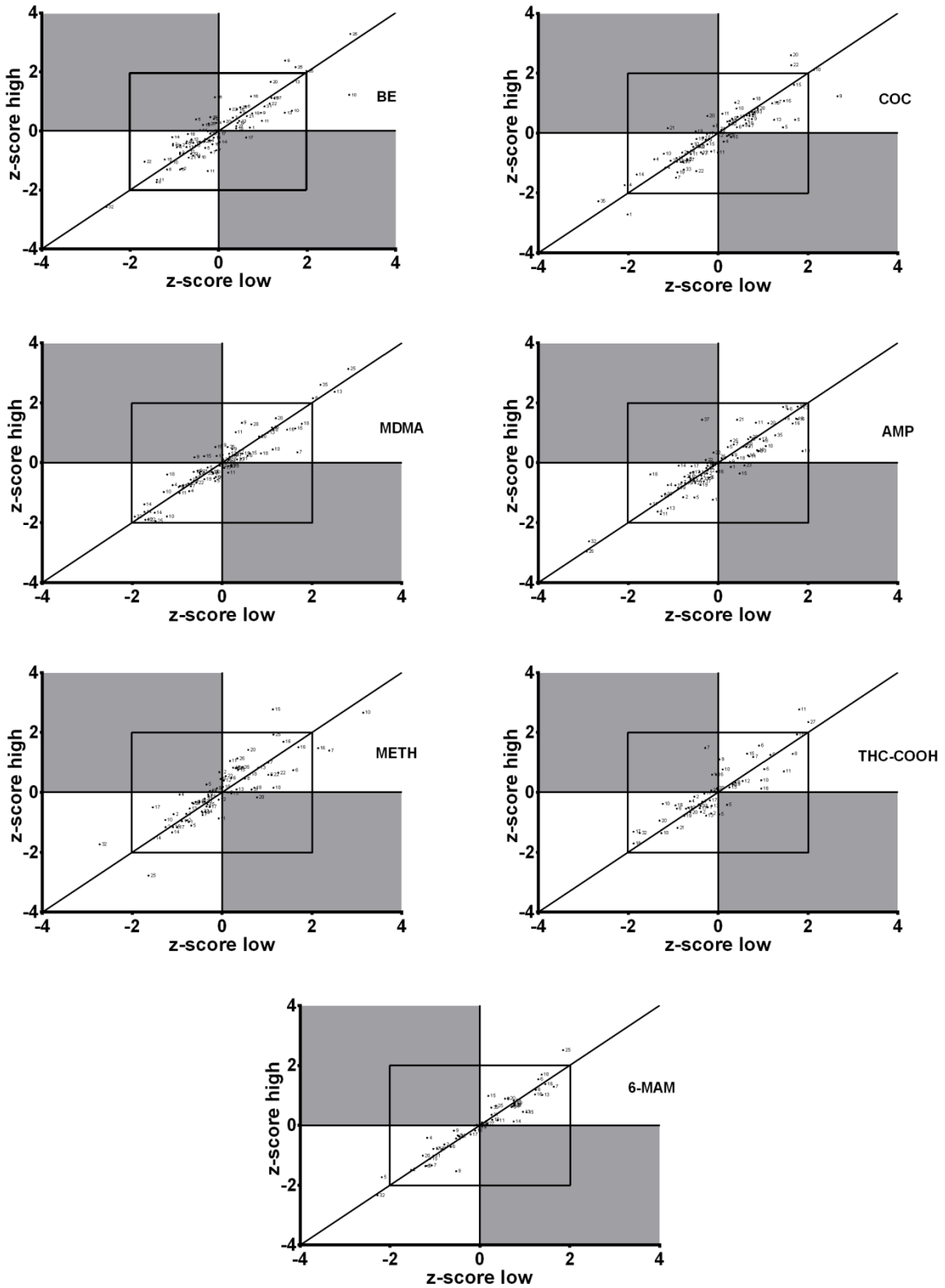


Figure 6. Percentage of participants with satisfactory results ($|z| \leq 2$) for tap water samples spiked with seven analytes. The dotted line represents 90% satisfactory level.



479

480 Figure 7. Youden plots with z-scores of the low concentration value (x-axis) and the z-scores of the
 481 high concentration value (y-axis) for the seven analytes in tap water across the years. Each
 482 participant is presented by a unique number. The inner rectangle captures satisfactory z-scores.

Table 1. Overview of inter-laboratory exercises and the number of participants from 2011-2016. For the wastewater samples, the 'x' represents unknown background concentrations. L = concentration level; P = amount of participants.

		2011		2012		2013		2014		2015		2016		
		L	P	L	P	L	P	L	P	L	P	L	P	
Module 1	Standard solution in methanol (concentrations in ng/mL)	BE	50; 500	12	73; 117	13	500	15	500	21	25	26	30	26
		COC	50; 500	12	36; 222	13	400	15	600	20	40	25	25	25
		MDMA	50; 500	12	120; 147	12	800	15	900	21	60	26	20	26
		AMP	50; 500	12	56; 132	13	700	15	750	21	120	26	40	26
		METH	50; 500	12	128; 134	13	200	15	150	21	80	26	50	26
		THC-COOH	50; 500	10	226; 227	12	1000	13	1000	19	200	23	125	20
		6-MAM	50; 500	11	56; 66	8	300	10	250	15	180	19	60	18
Module 2	Tap water (concentrations in ng/L)	BE					40; 150	15	30; 120	20	30; 80; 140	23	10; 65; 130	26
		COC					50; 100	15	60; 150	20	60; 100; 150	23	5; 50; 100	25
		MDMA					90; 300	15	80; 400	20	90; 120; 260	23	8; 75; 150	26
		AMP					80; 250	15	70; 200	20	80; 160; 200	23	12; 70; 140	26
		METH					10; 50	15	25; 100	20	50; 90; 180	23	6; 60; 120	26
		THC-COOH					100; 400	11	200; 500	16	250; 350; 450	20	50; 150; 300	20
		6-MAM					30; 90	10	90; 180	14	150; 210; 300	17	5; 80; 160	18
	Wastewater (concentrations in ng/L)	BE			x; x+16	13	x+40; x+150	15	x+30; x+120	19				
		COC			x; x+8	13	x+50; x+100	15	x+60; x+150	19				
		MDMA			x; x+42	13	x+90; x+300	15	x+80; x+400	20				
		AMP			x; x+118	13	x+80; x+250	15	x+70; x+200	20				
		METH			x; x+49	13	x+10; x+50	15	x+25; x+100	20				
		THC-COOH			x; x+75	12	x+100; x+400	10	x+200; x+500	17				
6-MAM			x; x+88	8	x+30; x+90	10	x+90; x+180	14						

Supporting Information for

Multi-year interlaboratory exercises for the analysis of illicit drugs and metabolites in wastewater: development of a quality control system

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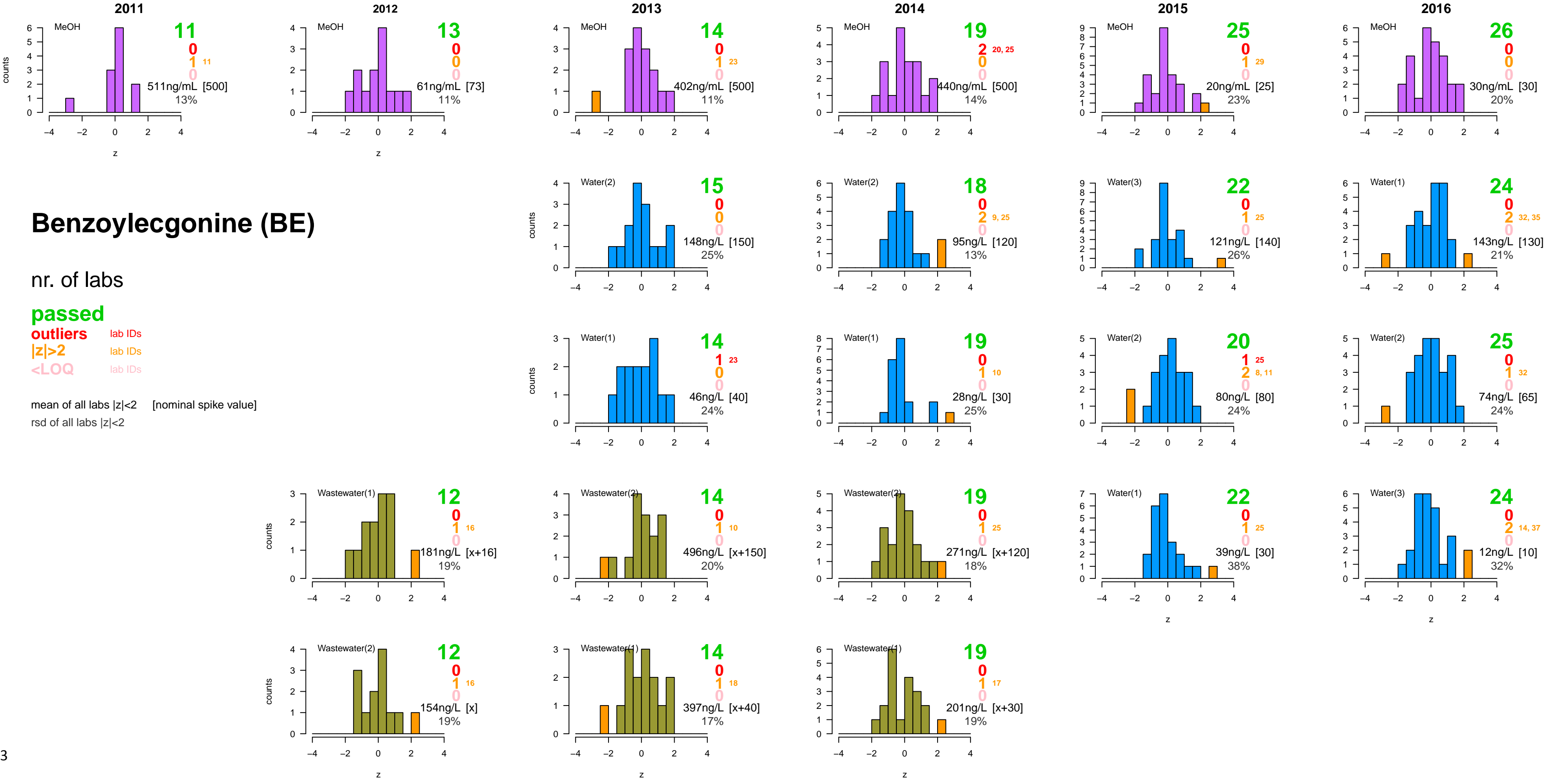
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Content

Substance	Overviews	Details
Benzoylcgonine (BE).....	p3	MeOH..... p10 Water (W) p11 Wastewater (WW) p12
Cocaine (COC).....	p4	MeOH..... p13 Water (W) p14 Wastewater (WW) p15
MDMA.....	p5	MeOH..... p16 Water (W) p17 Wastewater (WW) p18
Amphetamine (AMP)	p6	MeOH..... p19 Water (W) p20 Wastewater (WW) p21
Methamphetamine (METH)	p7	MeOH..... p22 Water (W) p23 Wastewater (WW) p24
THC-COOH.....	p8	MeOH..... p25 Water (W) p26 Wastewater (WW) p27
6-MAM	p9	MeOH..... p28 Water (W) p29 Wastewater (WW) p30



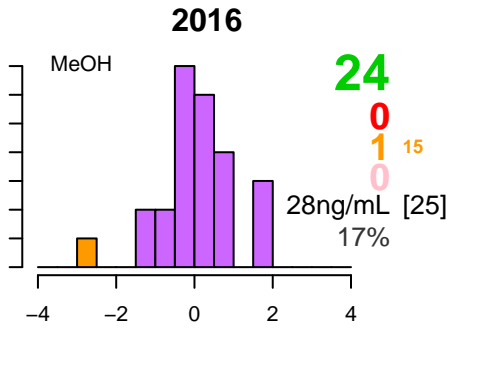
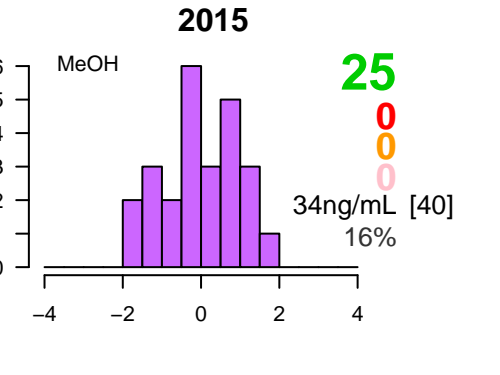
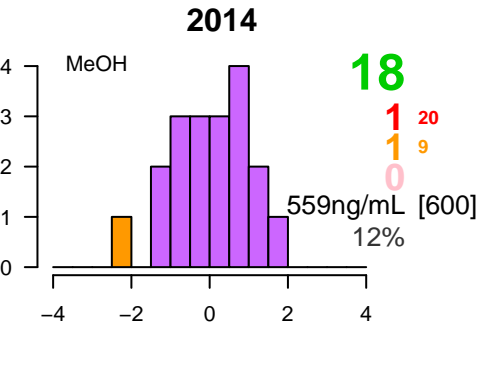
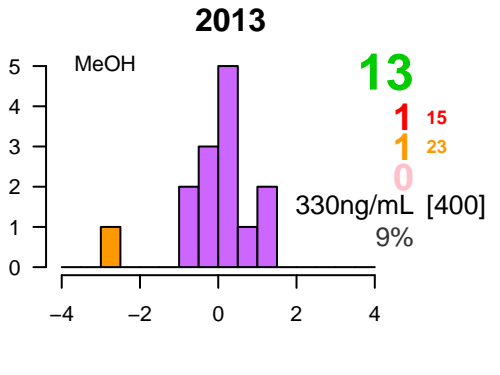
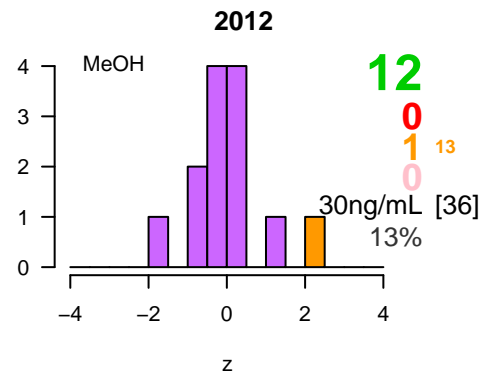
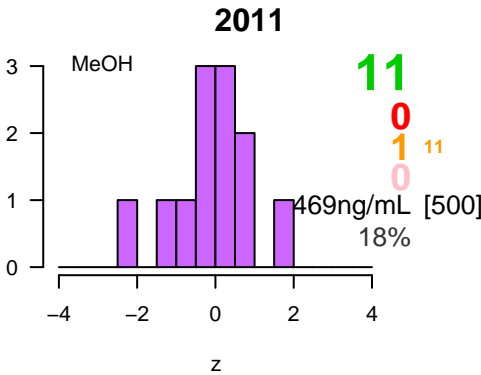
Benzoylecgonine (BE)

nr. of labs

passed

- outliers lab IDs
- |z|>2 lab IDs
- <LOQ lab IDs

mean of all labs |z|<2 [nominal spike value]
 rsd of all labs |z|<2



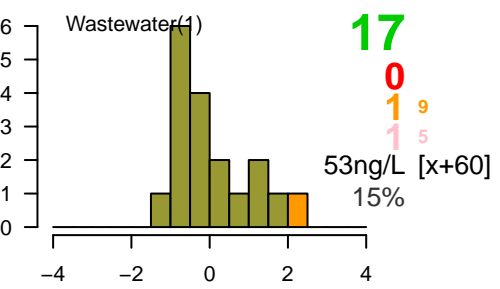
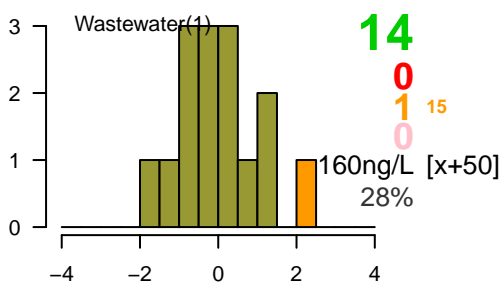
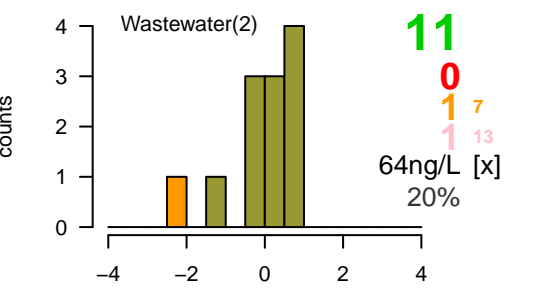
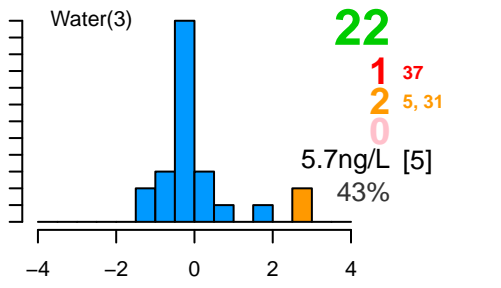
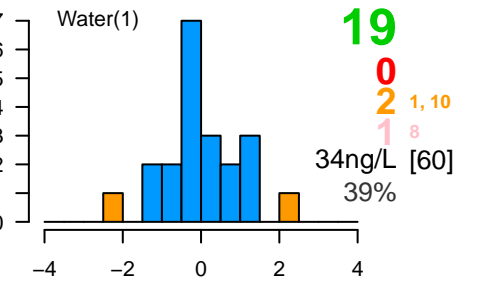
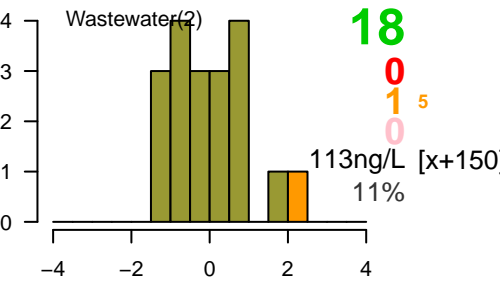
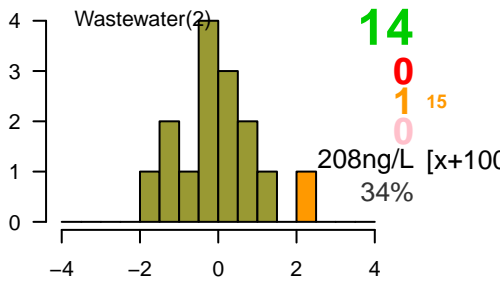
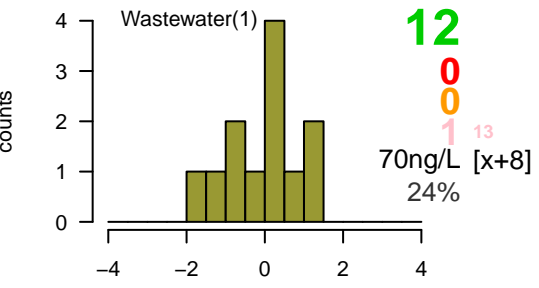
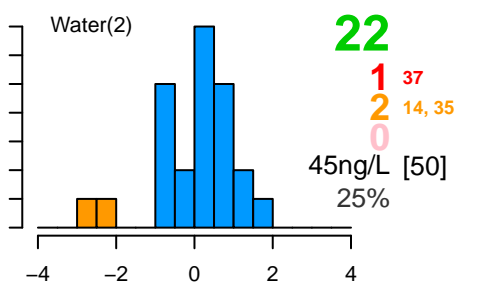
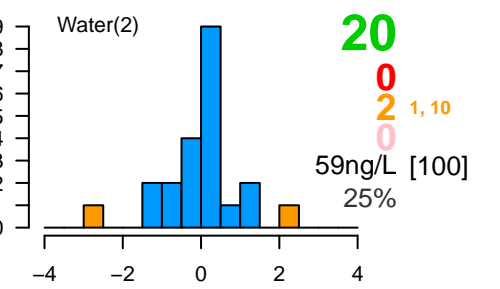
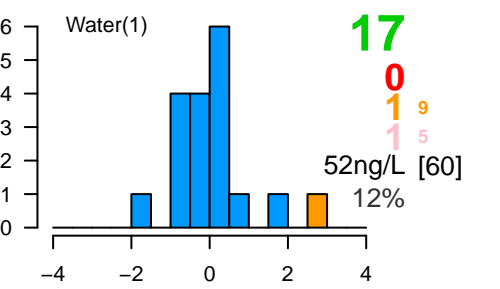
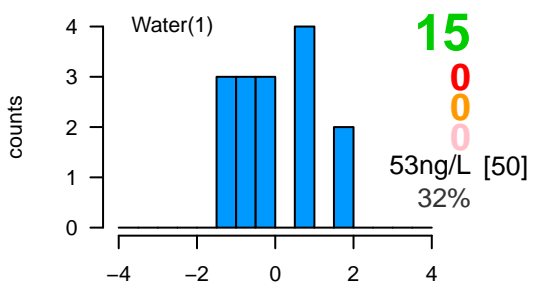
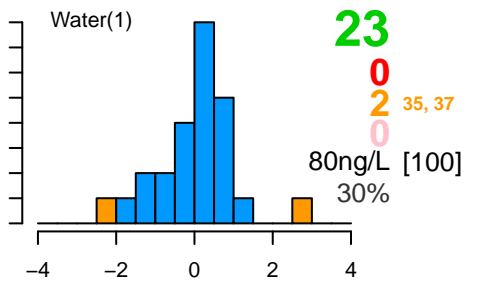
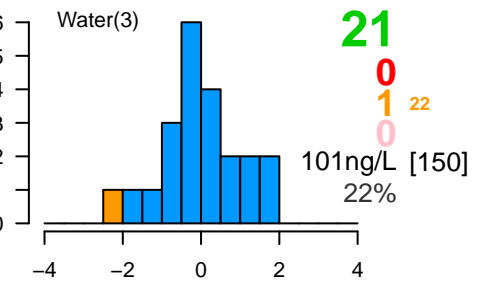
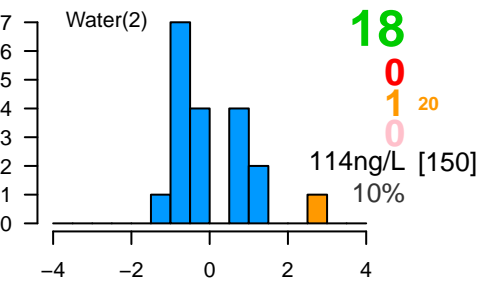
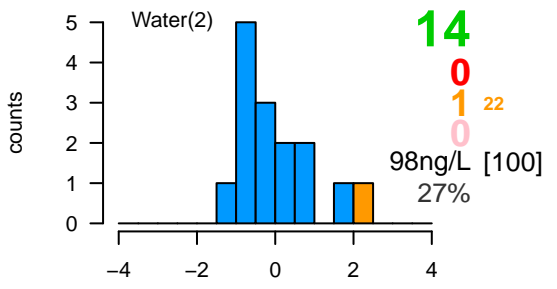
Cocaine (COC)

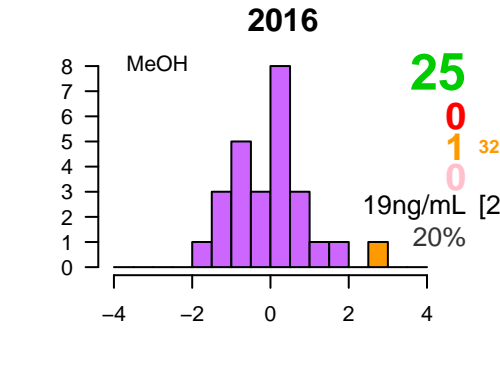
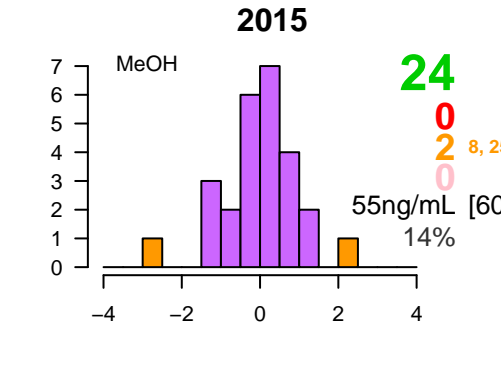
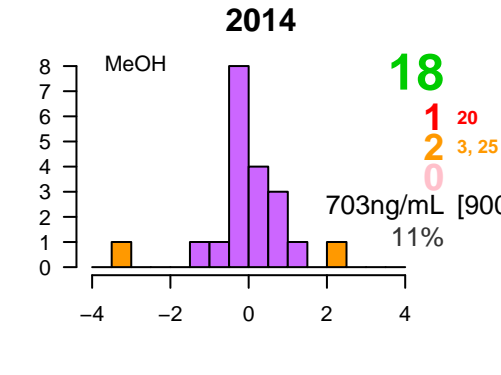
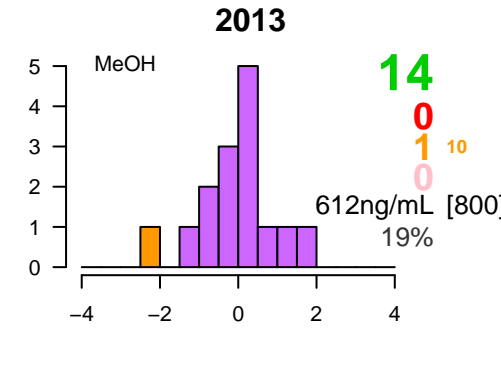
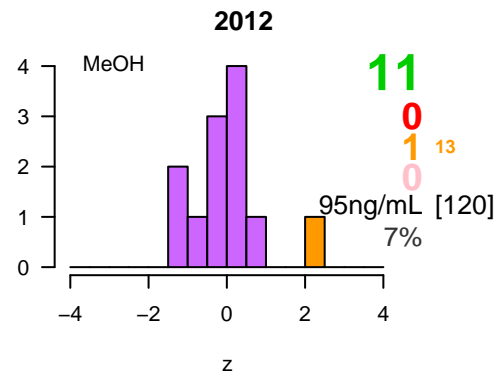
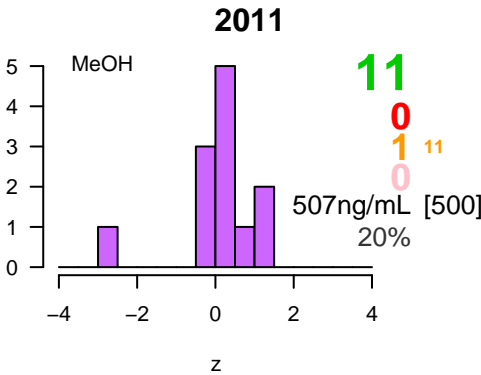
nr. of labs

passed

outliers lab IDs
 $|z| > 2$ lab IDs
 $< LOQ$ lab IDs

mean of all labs $|z| < 2$ [nominal spike value]
 rsd of all labs $|z| < 2$



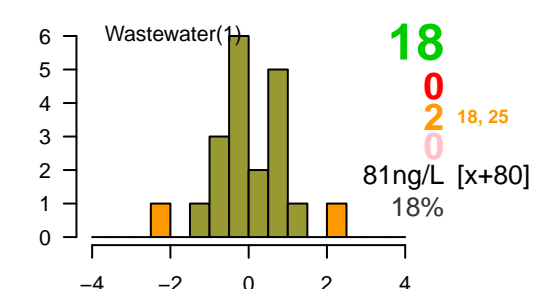
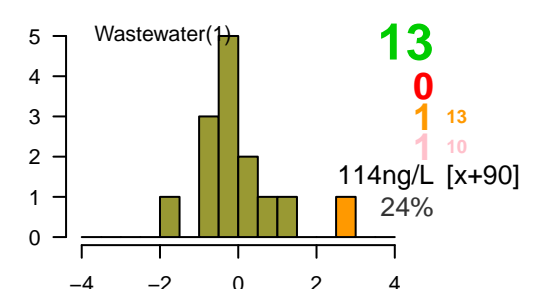
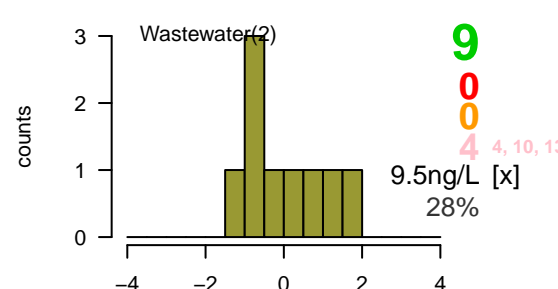
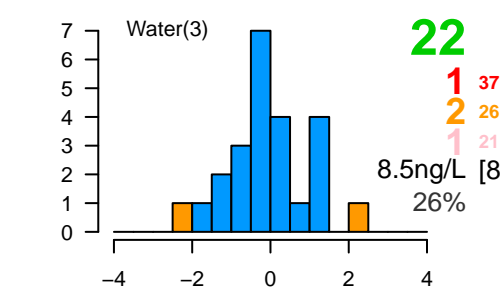
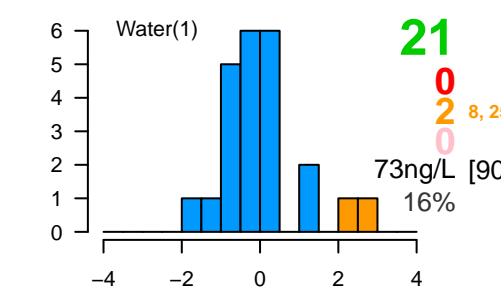
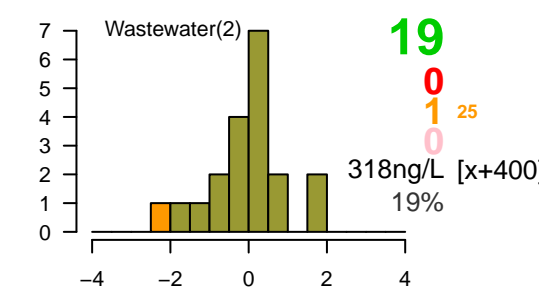
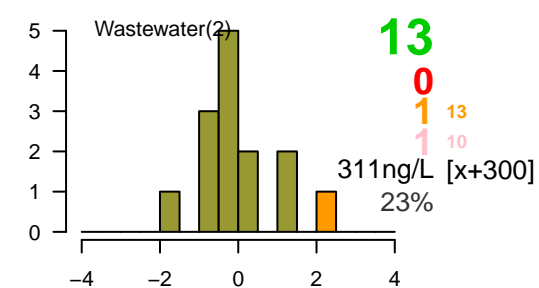
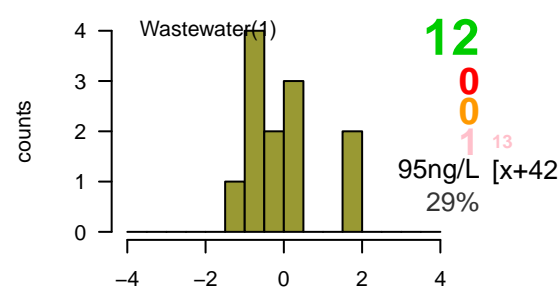
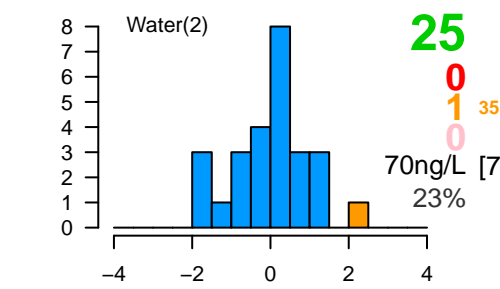
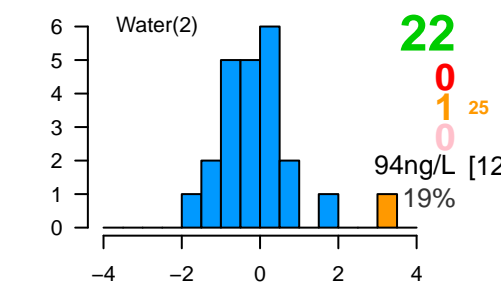
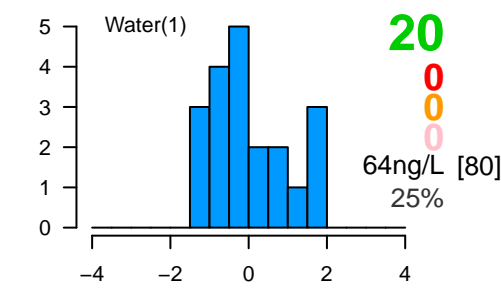
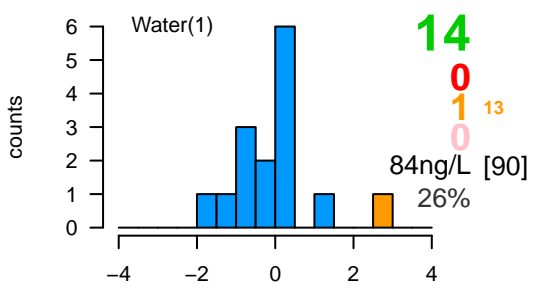
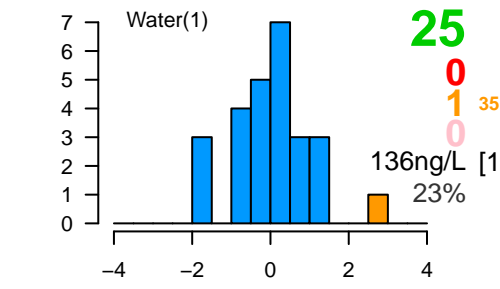
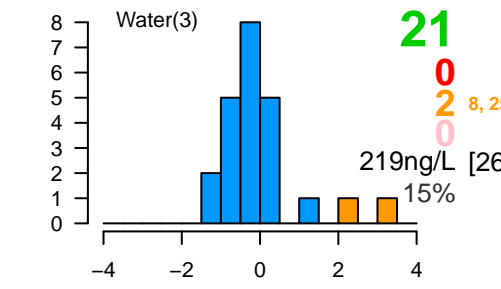
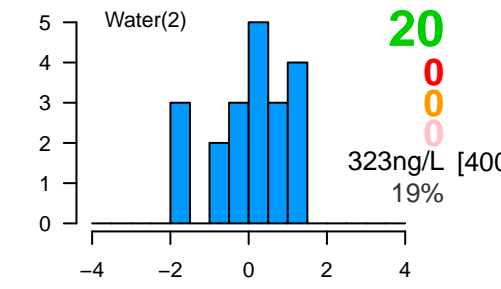
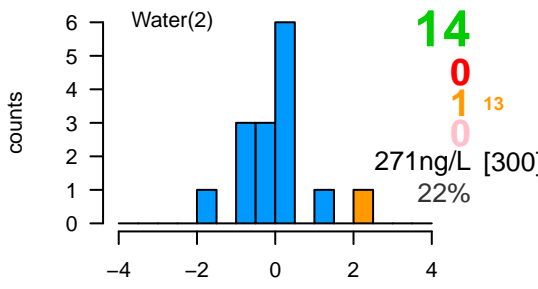


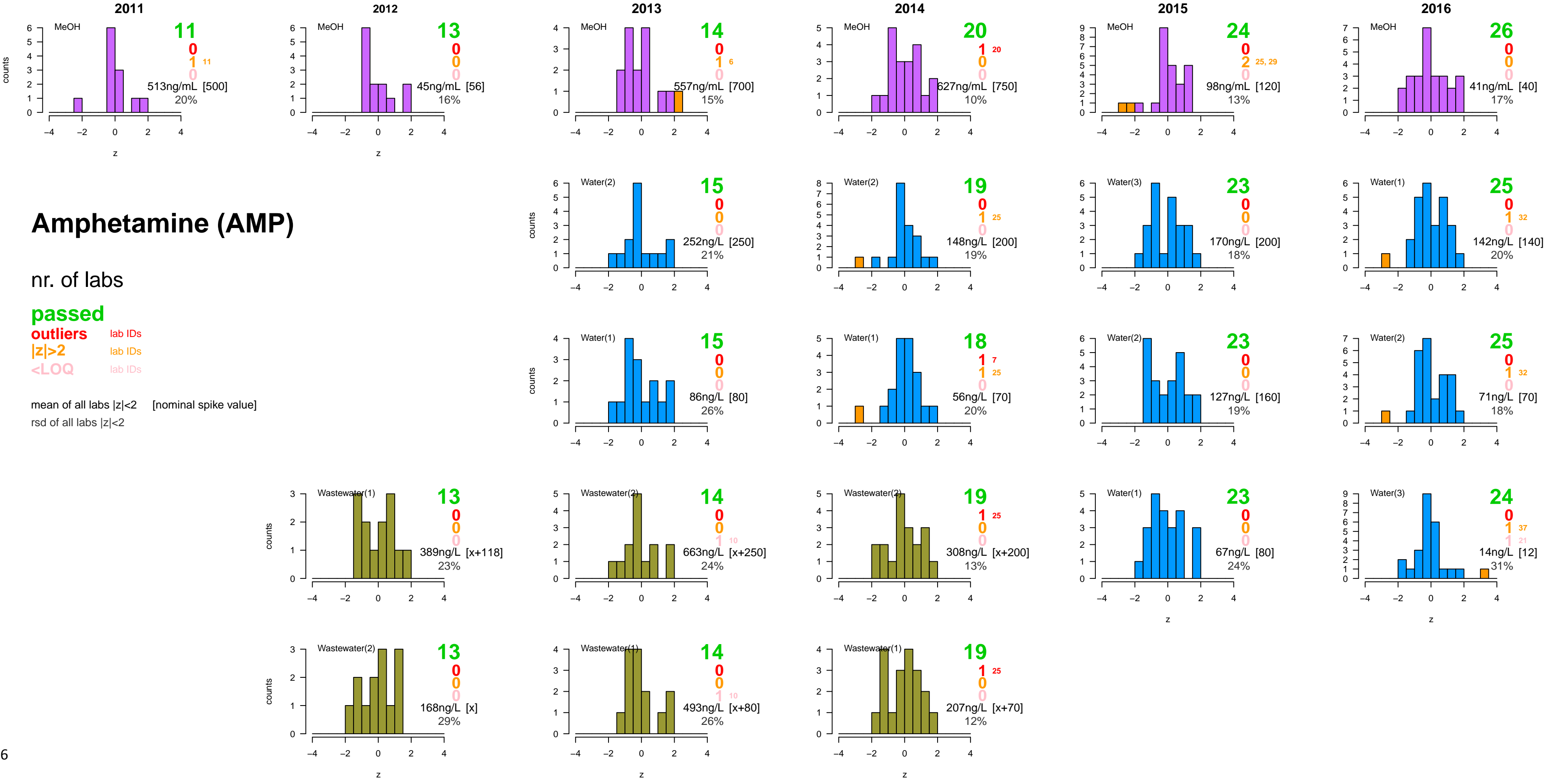
MDMA

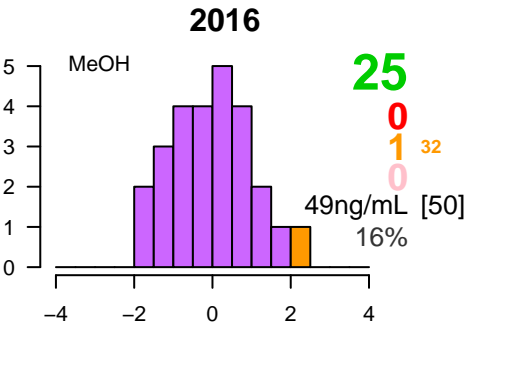
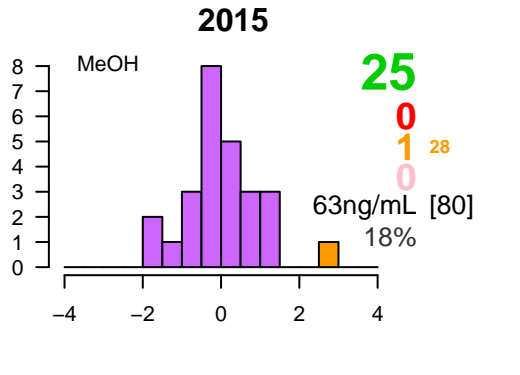
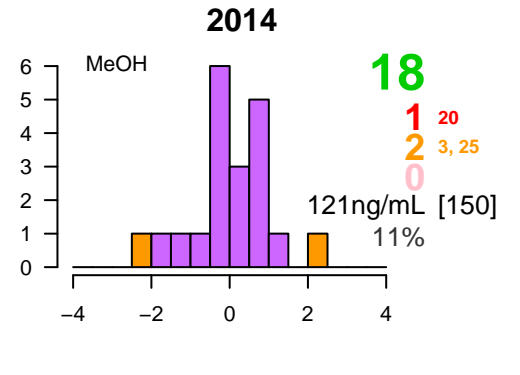
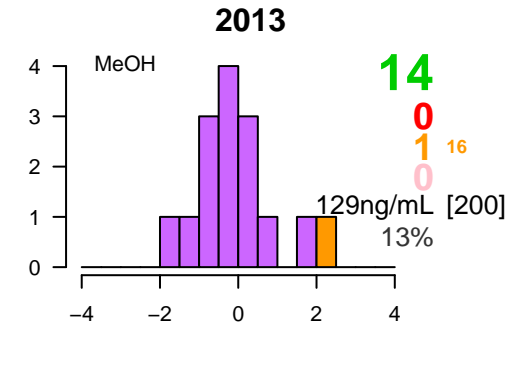
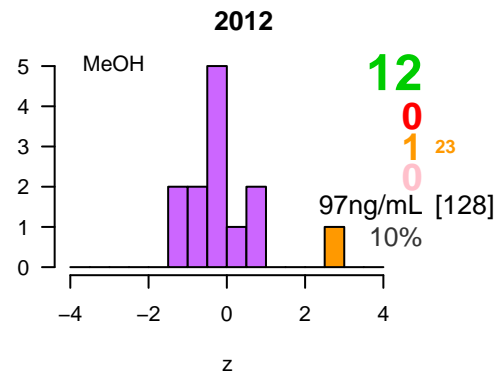
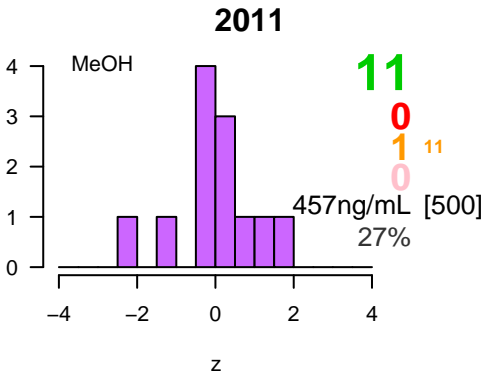
nr. of labs

- passed
- outliers lab IDs
- $|z| > 2$ lab IDs
- $< LOQ$ lab IDs

mean of all labs $|z| < 2$ [nominal spike value]
rsd of all labs $|z| < 2$







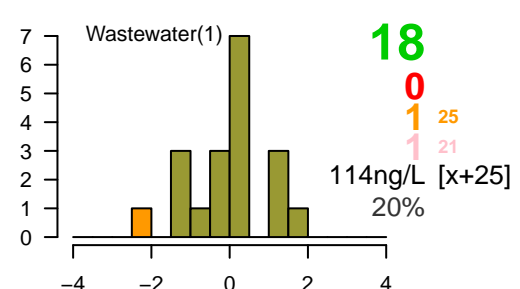
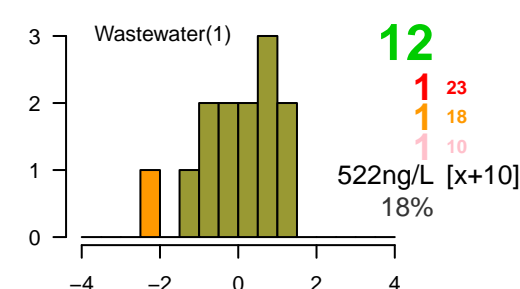
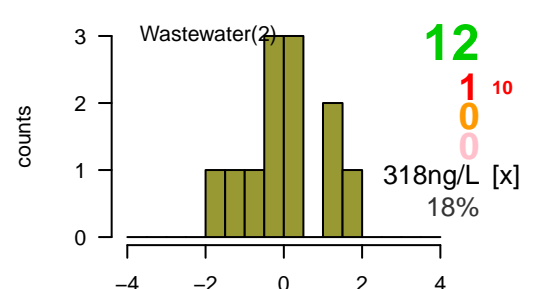
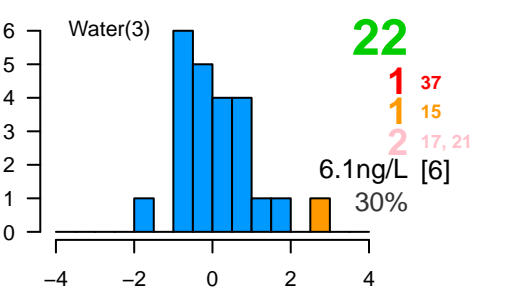
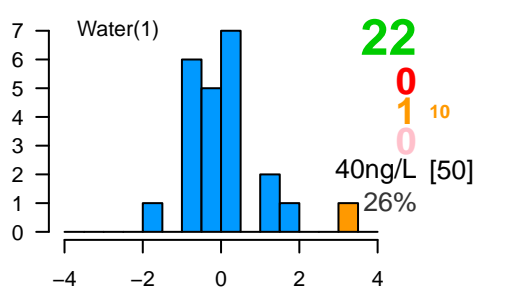
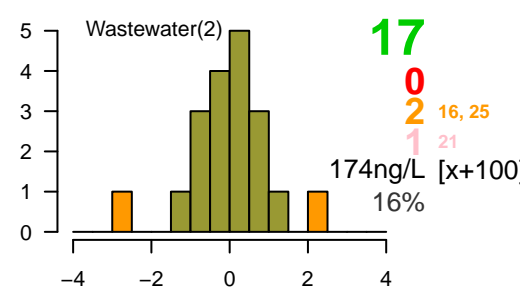
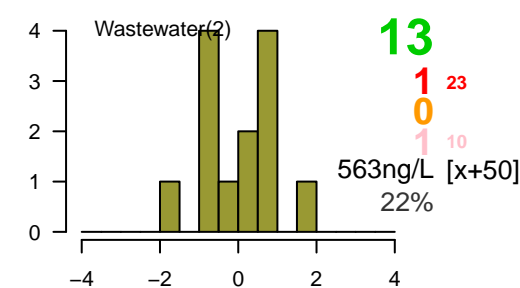
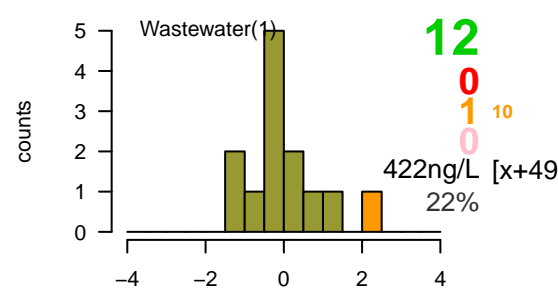
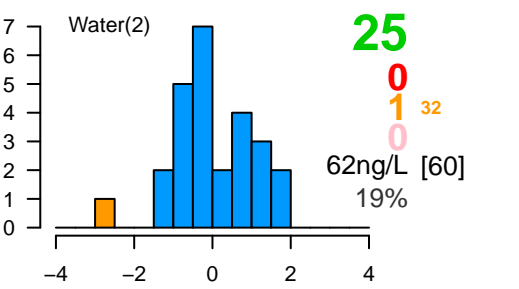
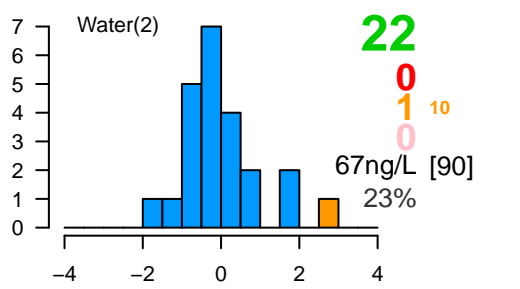
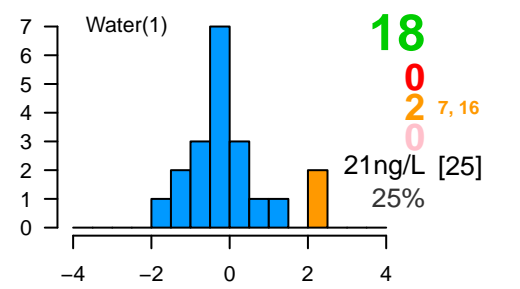
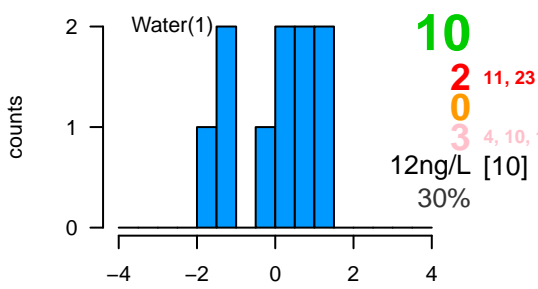
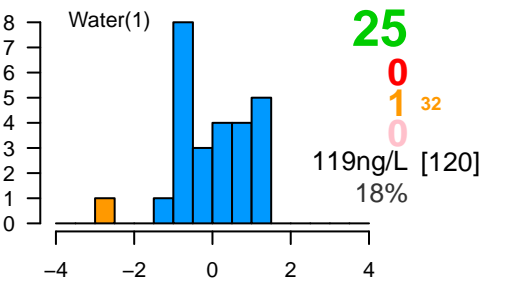
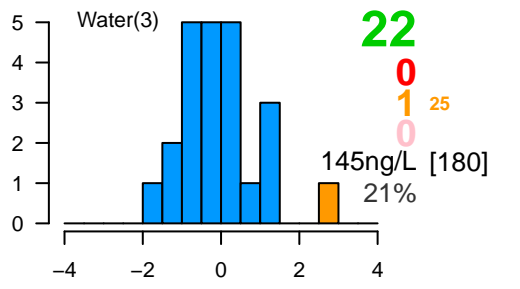
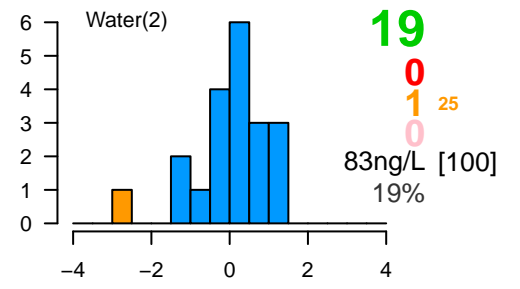
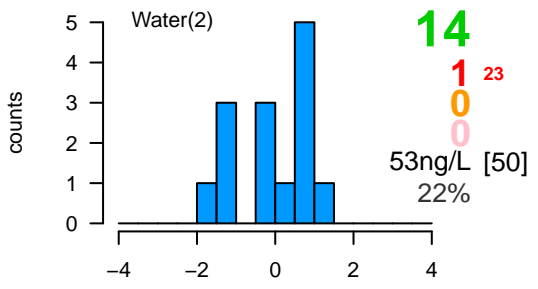
Methamphetamine (METH)

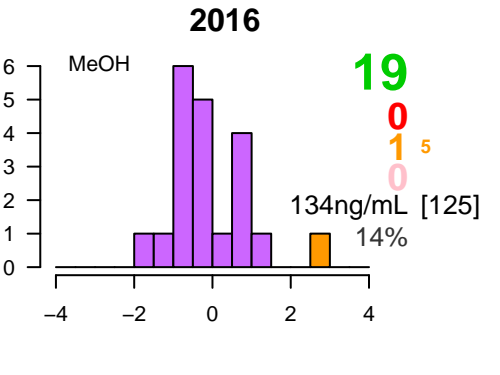
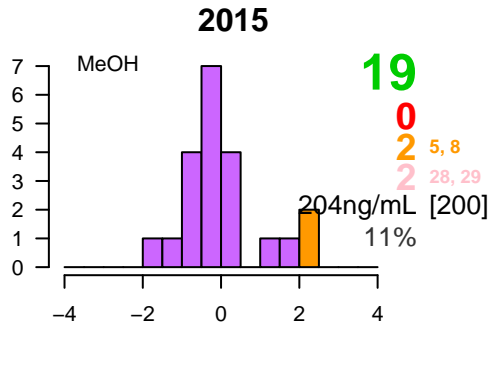
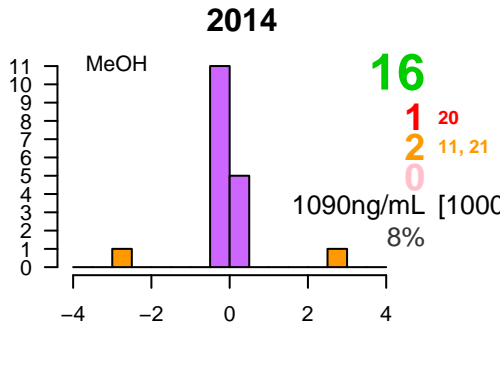
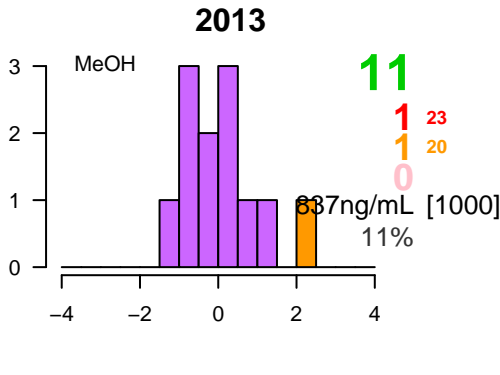
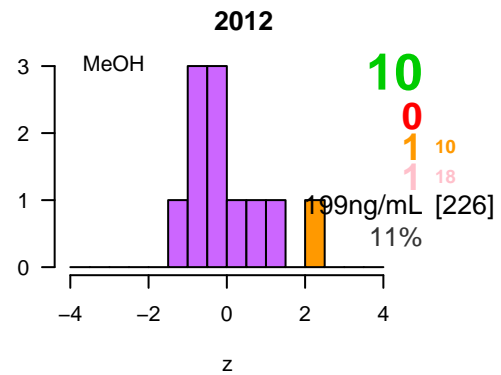
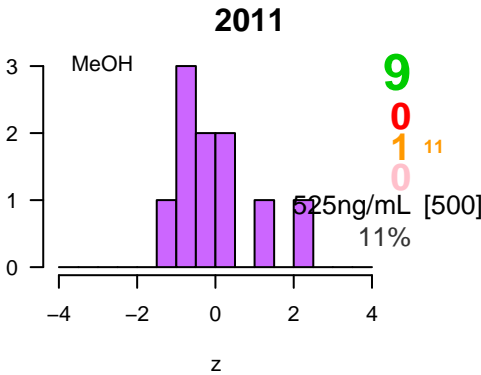
nr. of labs

passed

outliers lab IDs
|z|>2 lab IDs
<LOQ lab IDs

mean of all labs |z|<2 [nominal spike value]
rsd of all labs |z|<2





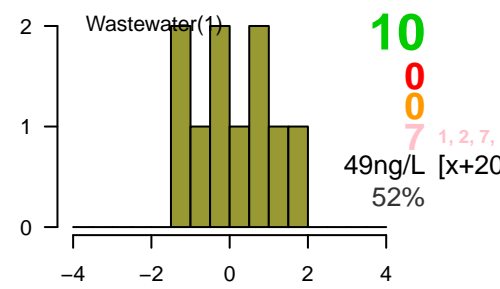
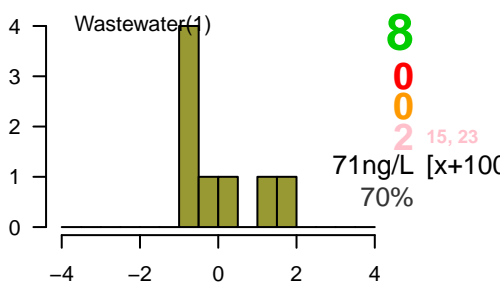
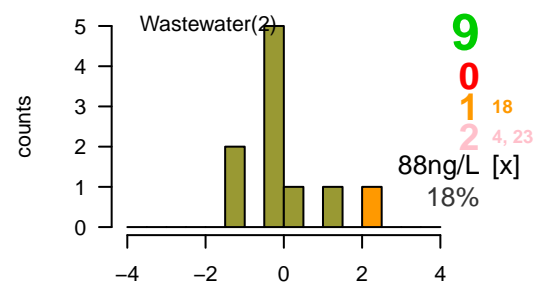
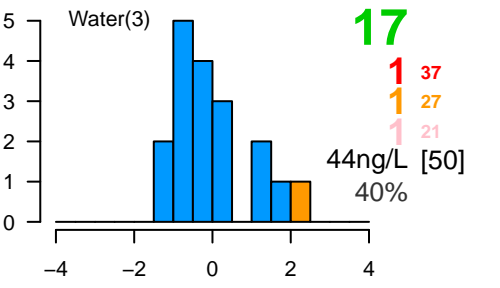
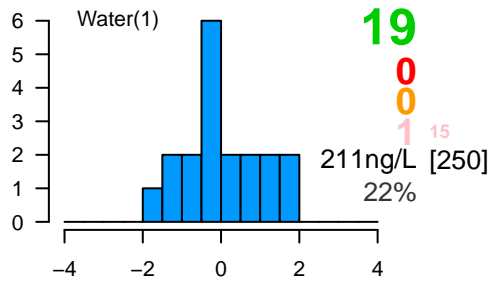
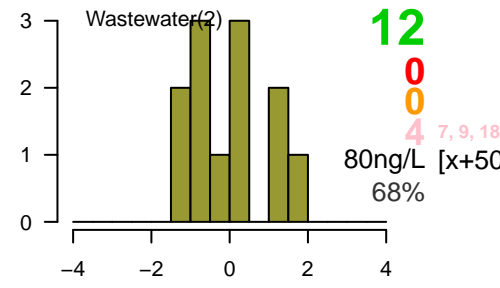
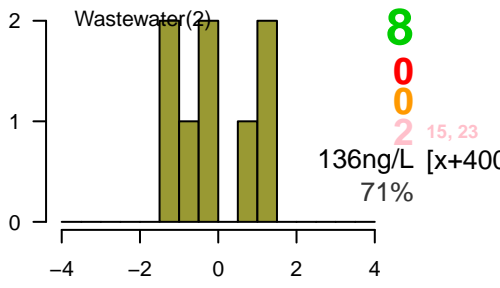
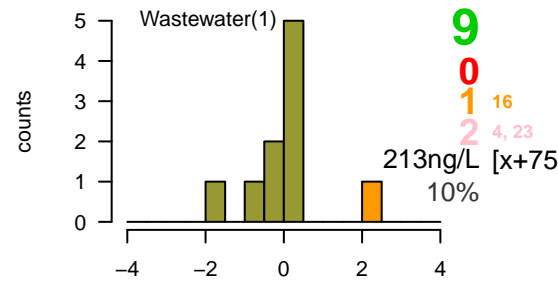
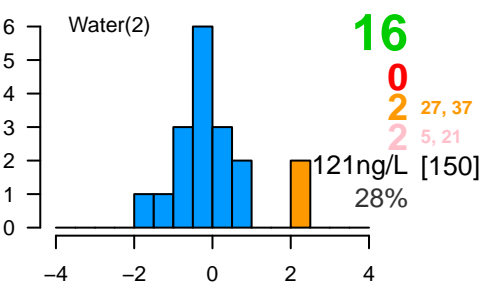
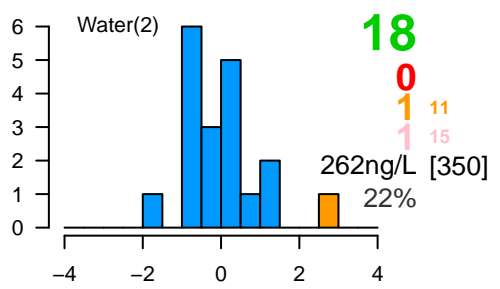
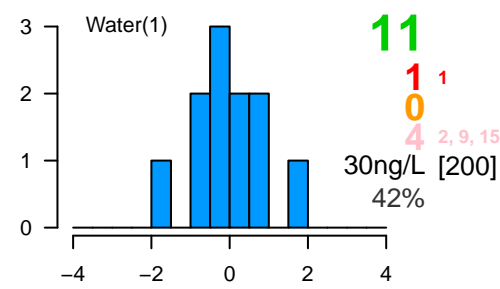
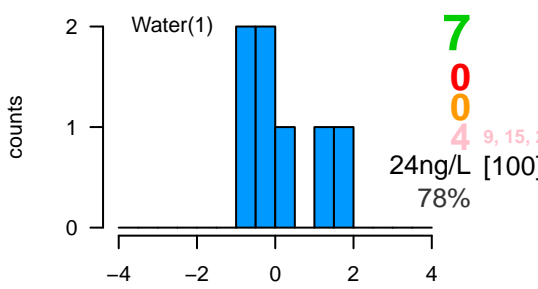
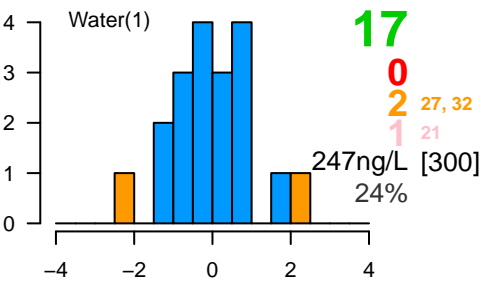
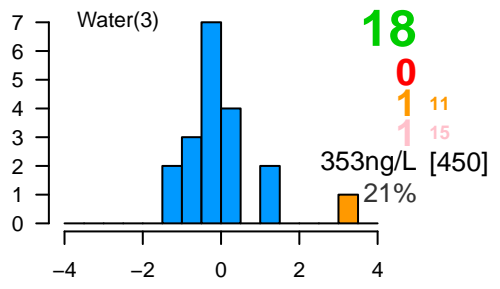
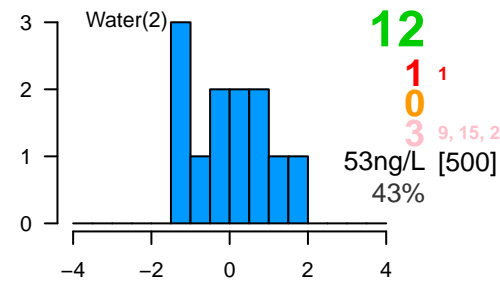
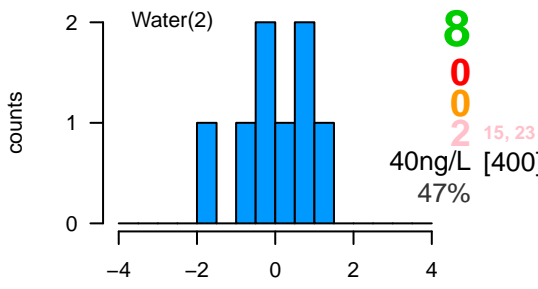
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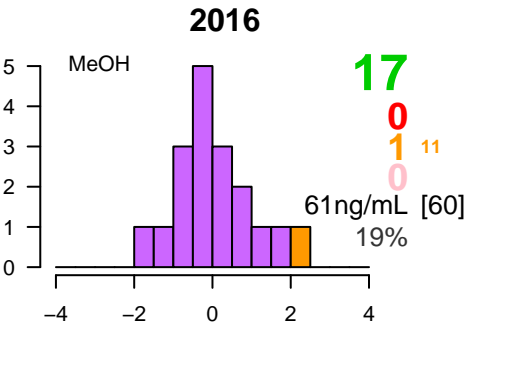
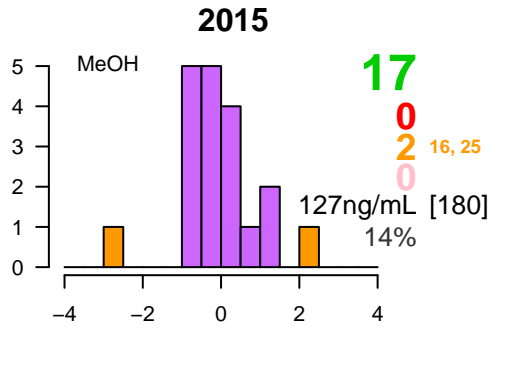
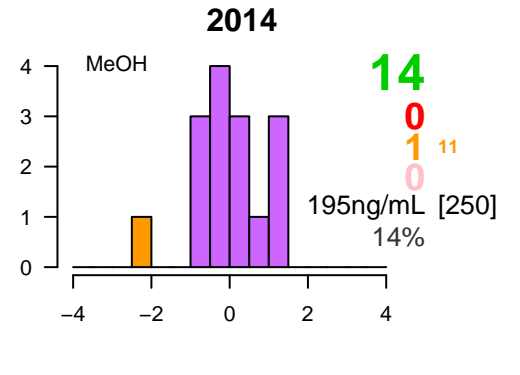
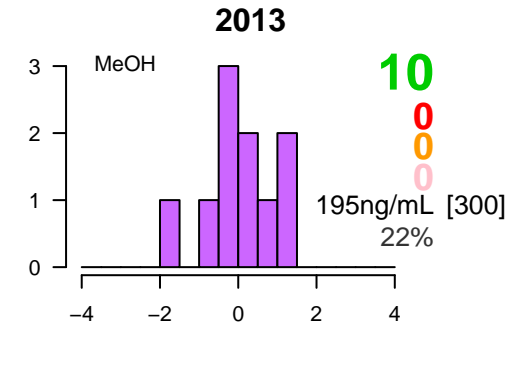
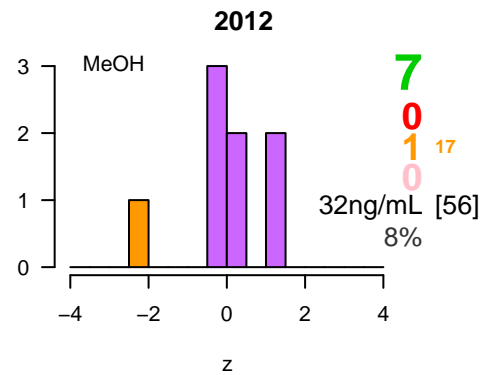
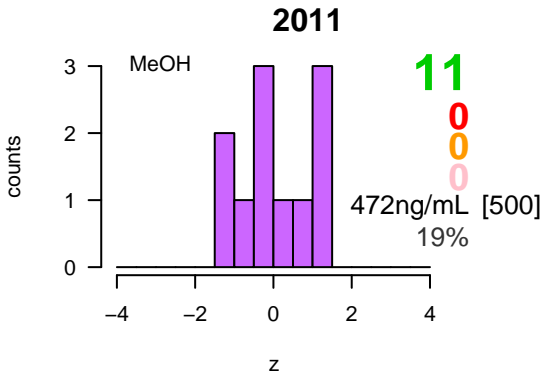
nr. of labs

passed

outliers lab IDs
|z|>2 lab IDs
<LOQ lab IDs

mean of all labs |z|<2 [nominal spike value]
rsd of all labs |z|<2



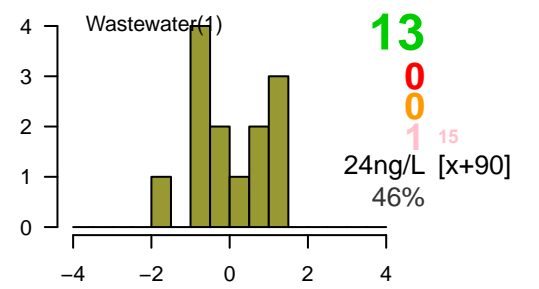
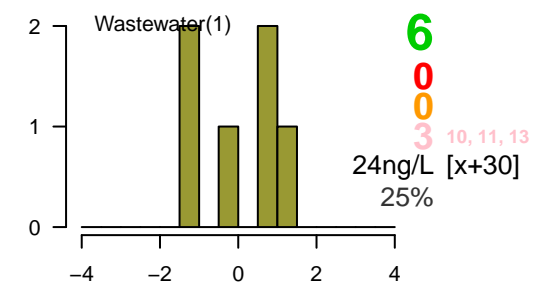
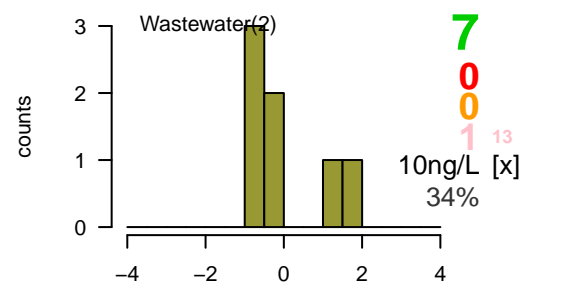
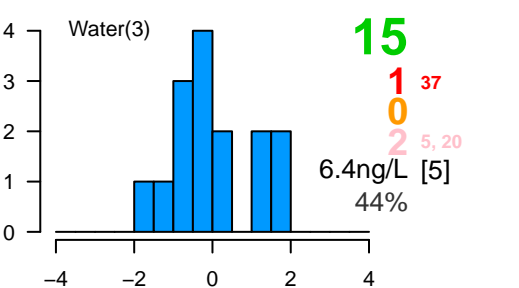
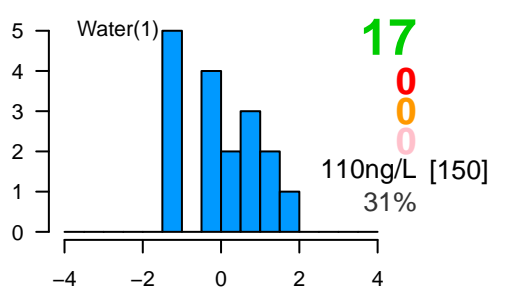
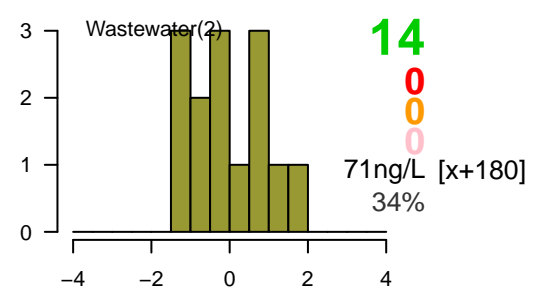
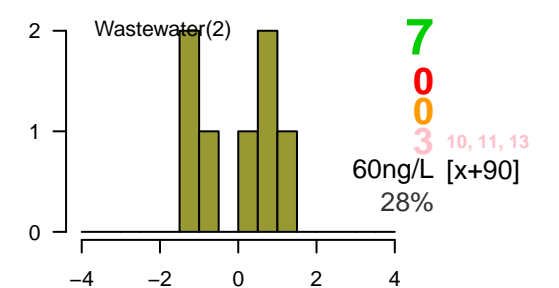
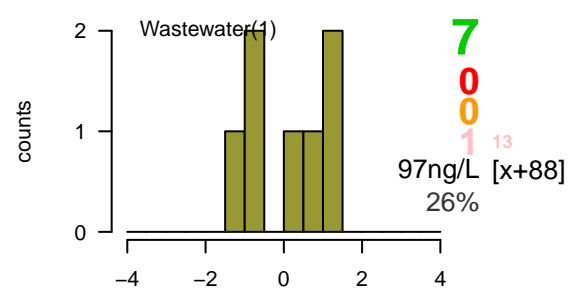
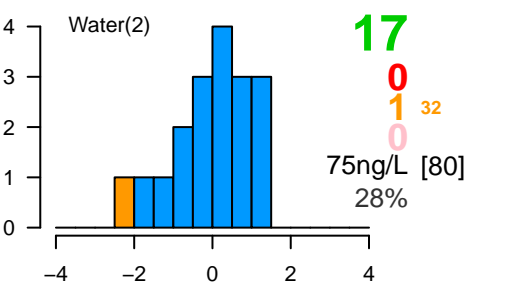
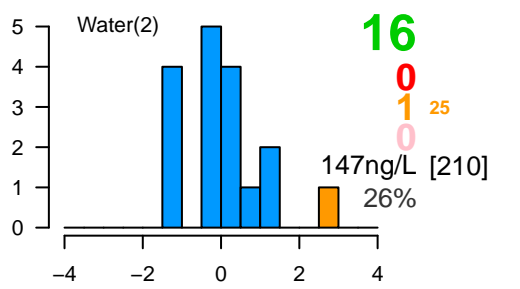
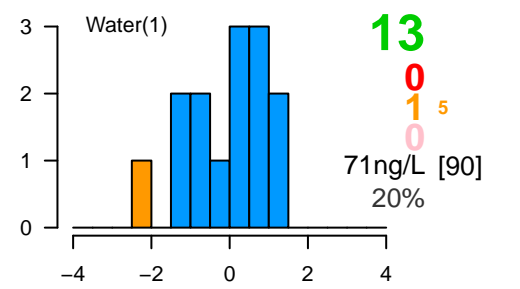
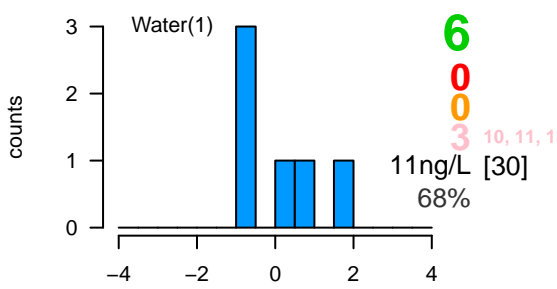
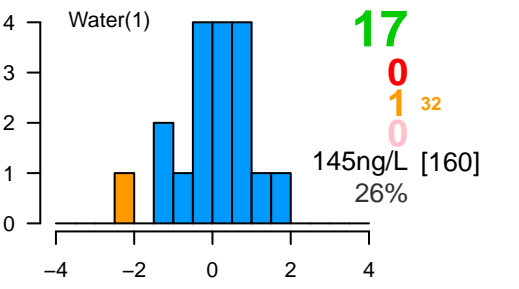
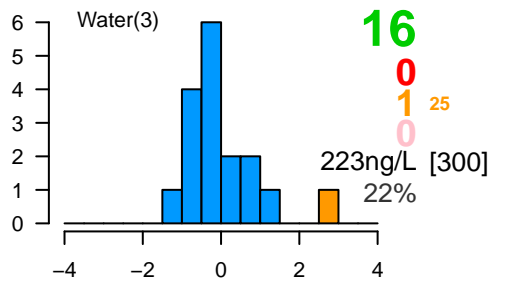
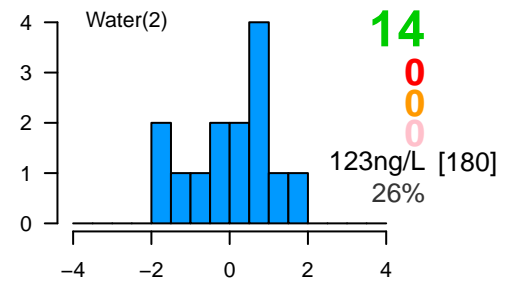
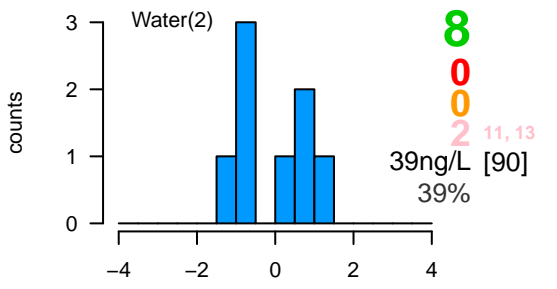


6-MAM

nr. of labs

passed
outliers lab IDs
 $|z| > 2$ lab IDs
<LOQ lab IDs

mean of all labs $|z| < 2$ [nominal spike value]
rsd of all labs $|z| < 2$



BE MeOH

normalized concentrations (with mean of means per year after removing outliers)

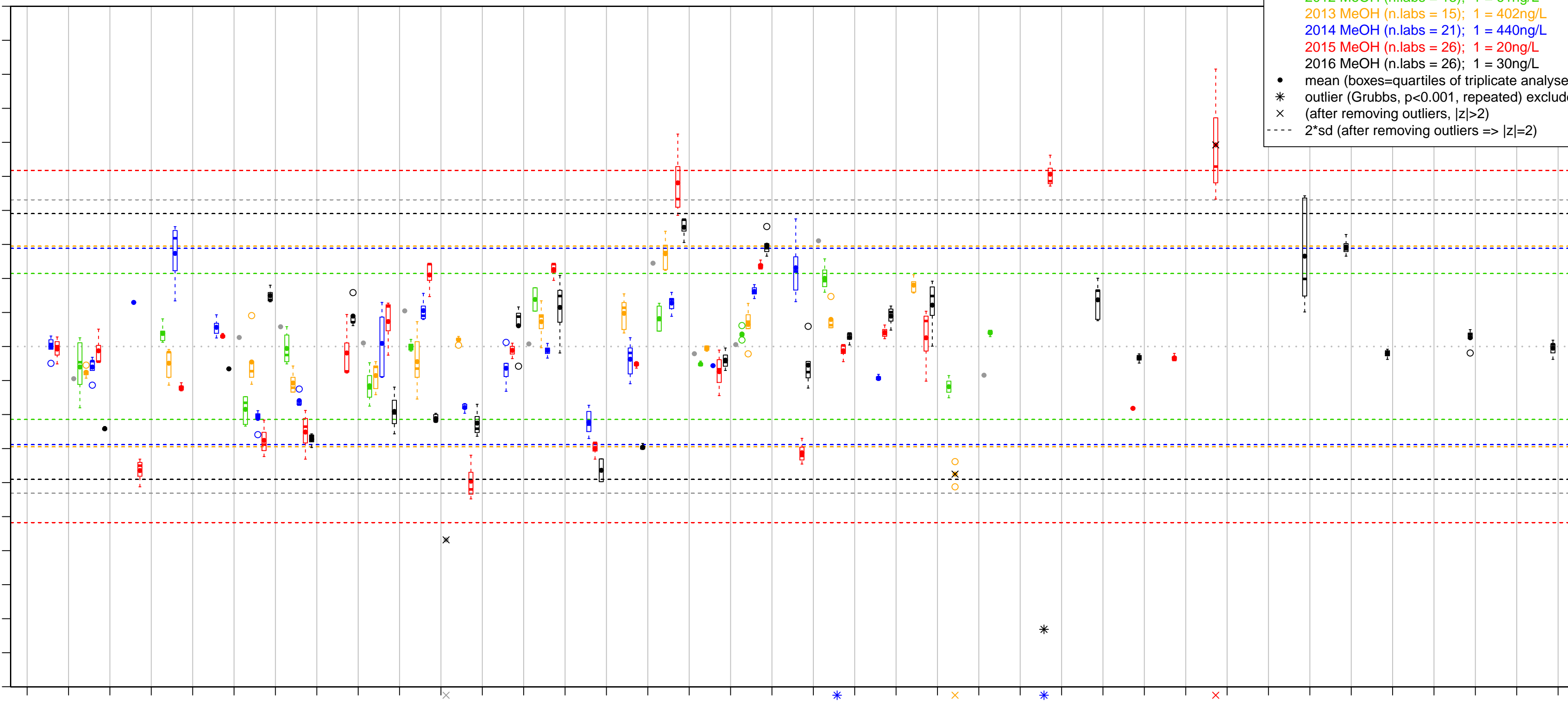
2.0
1.5
1.0
0.5
0.0

- 2011 MeOH (n.labs = 12); 1 = 511ng/L
- 2012 MeOH (n.labs = 13); 1 = 61ng/L
- 2013 MeOH (n.labs = 15); 1 = 402ng/L
- 2014 MeOH (n.labs = 21); 1 = 440ng/L
- 2015 MeOH (n.labs = 26); 1 = 20ng/L
- 2016 MeOH (n.labs = 26); 1 = 30ng/L
- mean (boxes=quartiles of triplicate analyses)
- * outlier (Grubbs, $p < 0.001$, repeated) excluded
- × (after removing outliers, $|z| > 2$)
- - - 2*sd (after removing outliers => $|z| = 2$)

p10

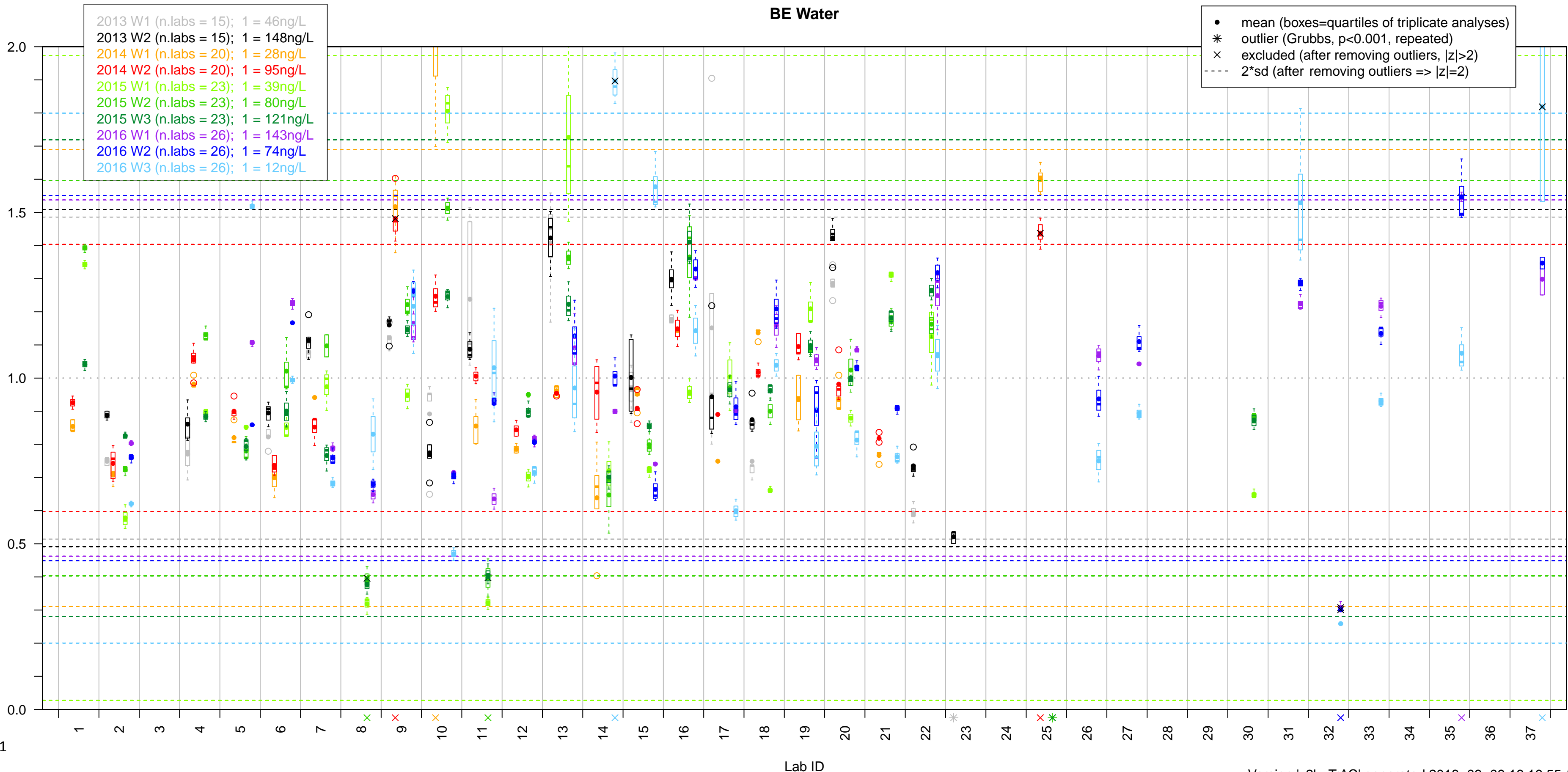
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37

Lab ID



BE Water

normalized concentrations (with mean of means per year after removing outliers)



p11

Lab ID

BE Wastewater

normalized concentrations (with mean of means per year after removing outliers)

2.0
1.5
1.0
0.5
0.0

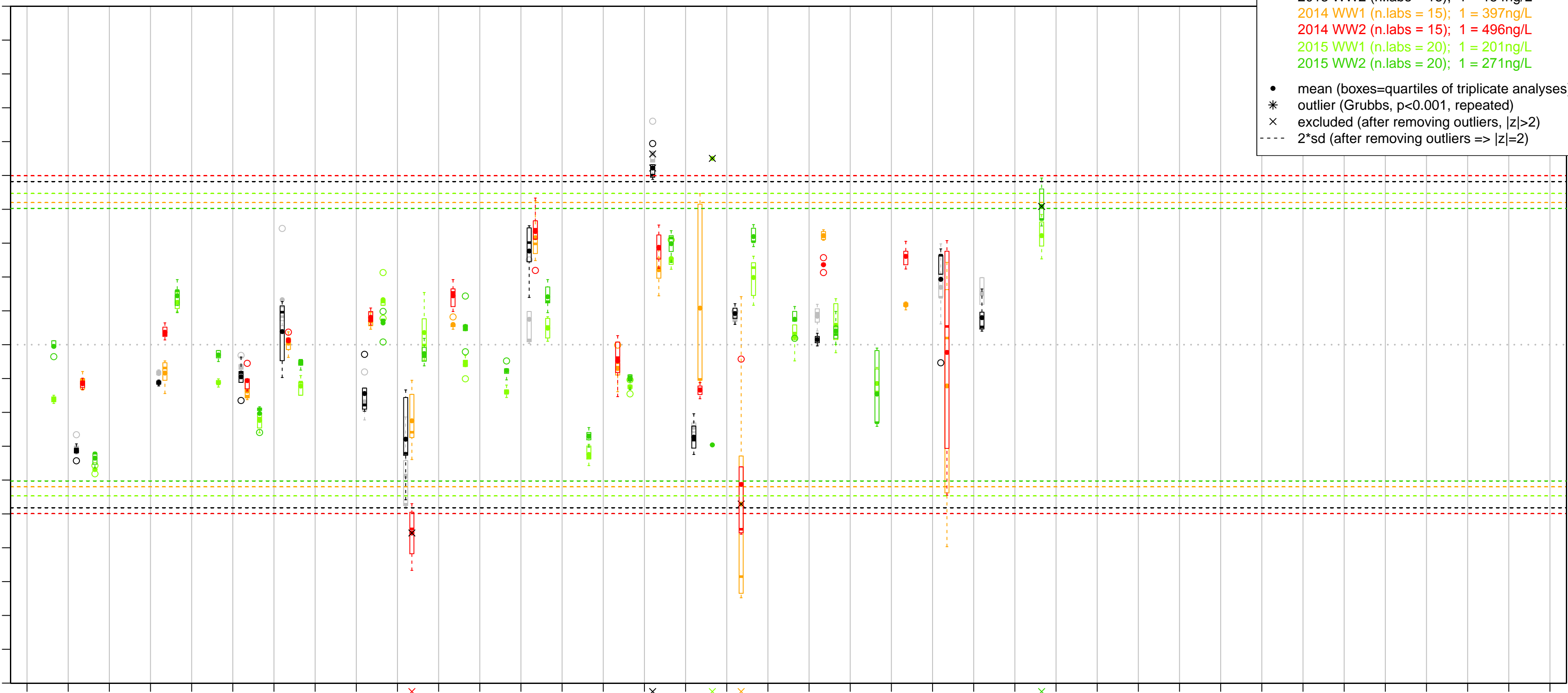
2013 WW1 (n.labs = 13); 1 = 181ng/L
2013 WW2 (n.labs = 13); 1 = 154ng/L
2014 WW1 (n.labs = 15); 1 = 397ng/L
2014 WW2 (n.labs = 15); 1 = 496ng/L
2015 WW1 (n.labs = 20); 1 = 201ng/L
2015 WW2 (n.labs = 20); 1 = 271ng/L

- mean (boxes=quartiles of triplicate analyses)
- * outlier (Grubbs, $p < 0.001$, repeated)
- × excluded (after removing outliers, $|z| > 2$)
- - - $2 \cdot sd$ (after removing outliers $\Rightarrow |z| = 2$)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37

Lab ID

p12



COC MeOH

normalized concentrations (with mean of means per year after removing outliers)

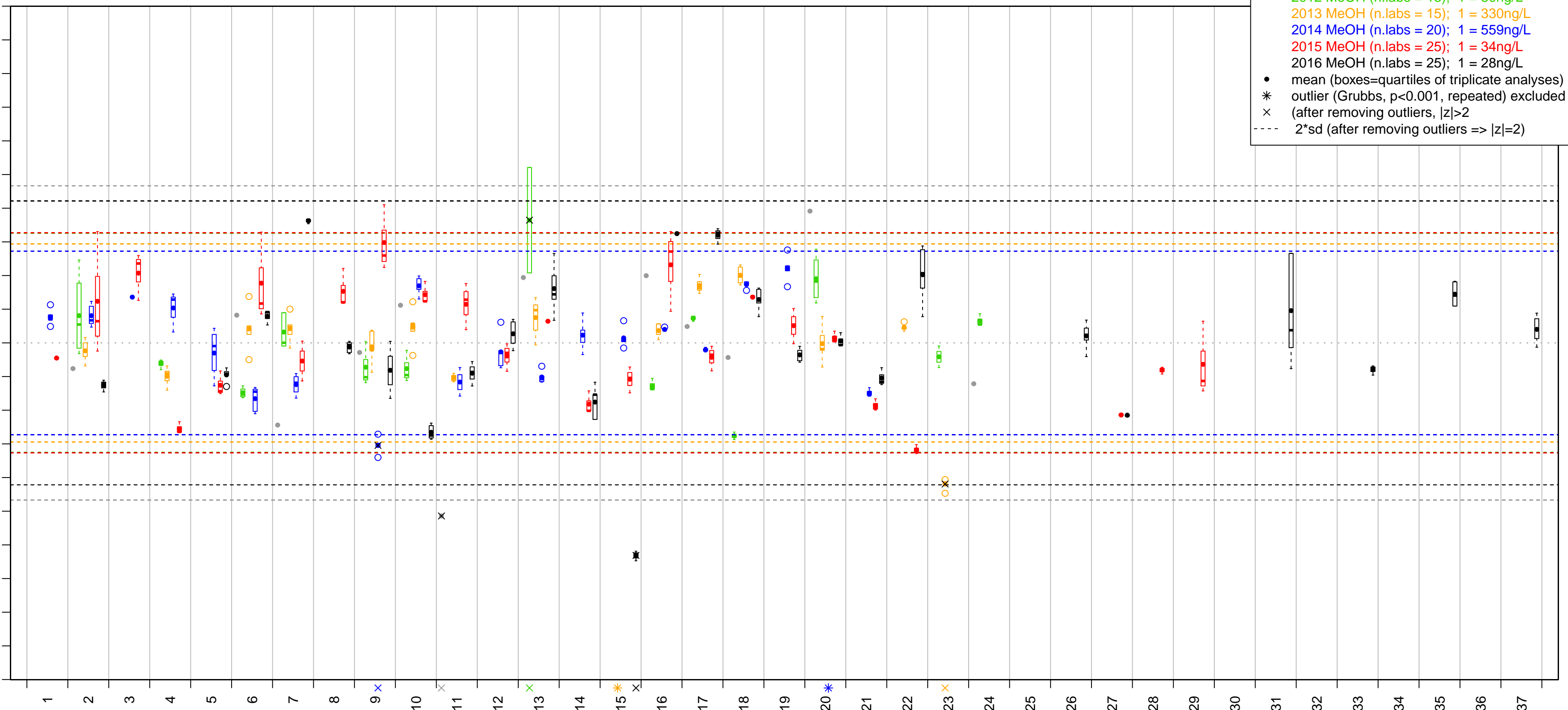
2.0
1.5
1.0
0.5
0.0

- 2011 MeOH (n.labs = 12); 1 = 469ng/L
- 2012 MeOH (n.labs = 13); 1 = 30ng/L
- 2013 MeOH (n.labs = 15); 1 = 330ng/L
- 2014 MeOH (n.labs = 20); 1 = 559ng/L
- 2015 MeOH (n.labs = 25); 1 = 34ng/L
- 2016 MeOH (n.labs = 25); 1 = 28ng/L
- mean (boxes=quartiles of triplicate analyses)
- * outlier (Grubbs, $p < 0.001$, repeated) excluded
- × (after removing outliers, $|z| > 2$)
- - - 2*sd (after removing outliers => $|z| = 2$)

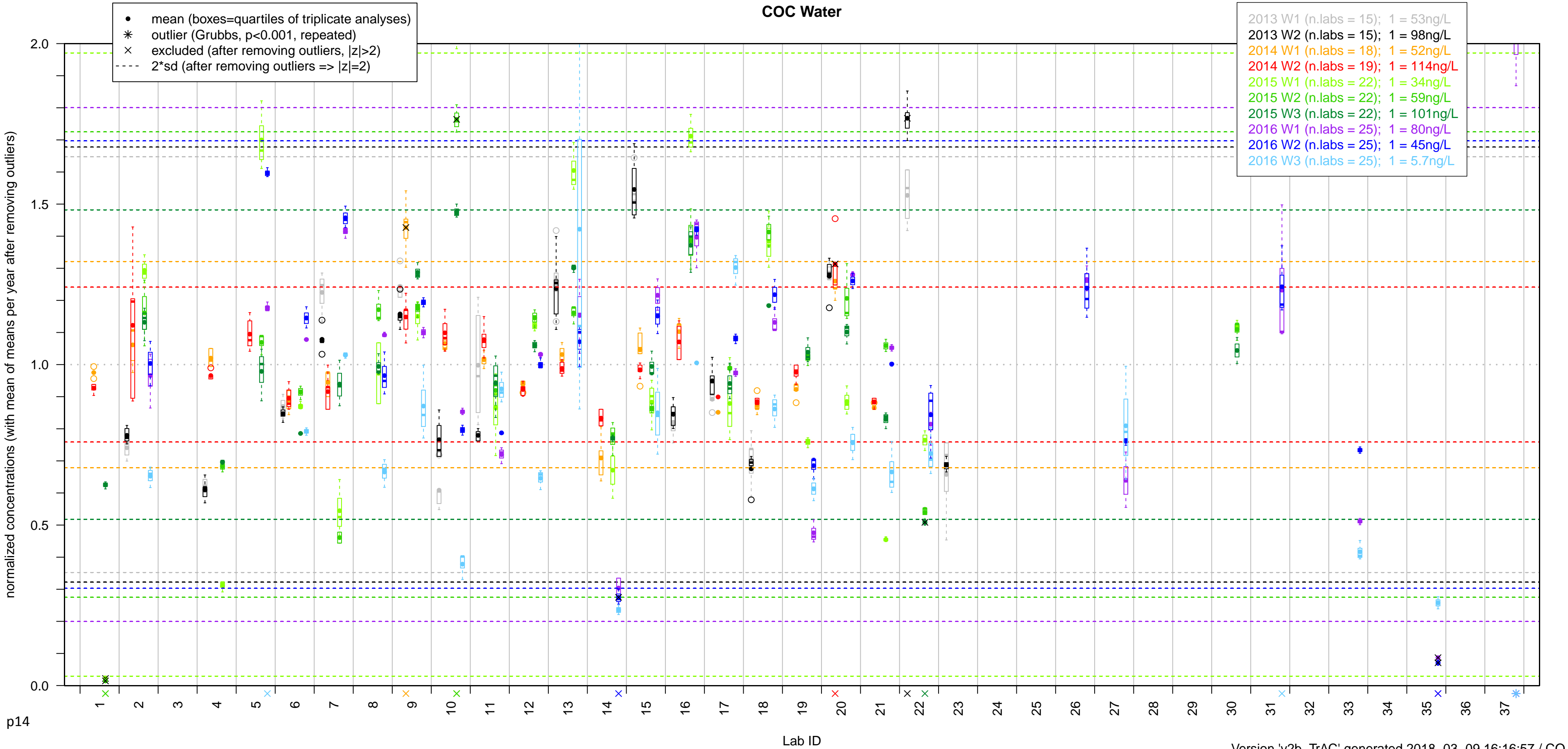
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37

Lab ID

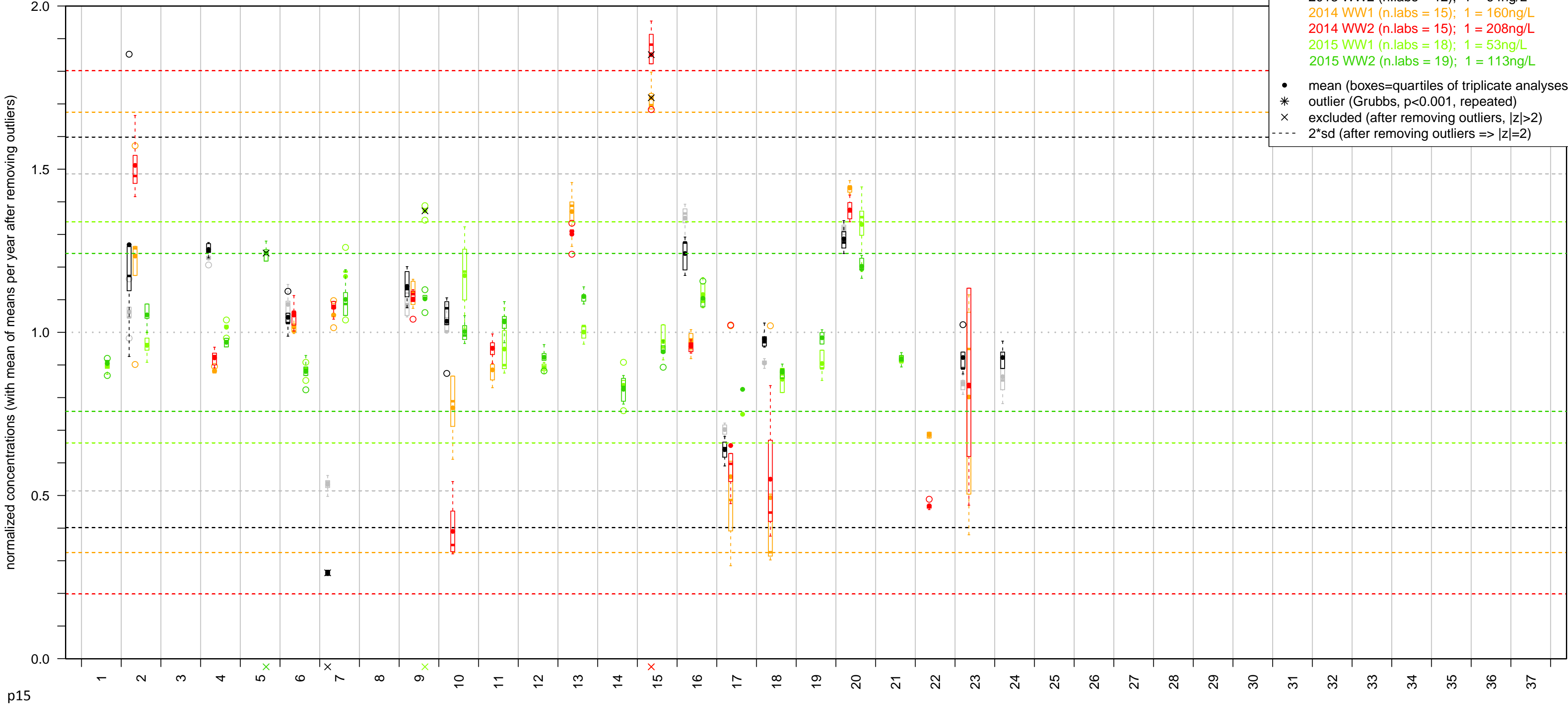
p13



COC Water



COC Wastewater



p15

MDMA MeOH

normalized concentrations (with mean of means per year after removing outliers)

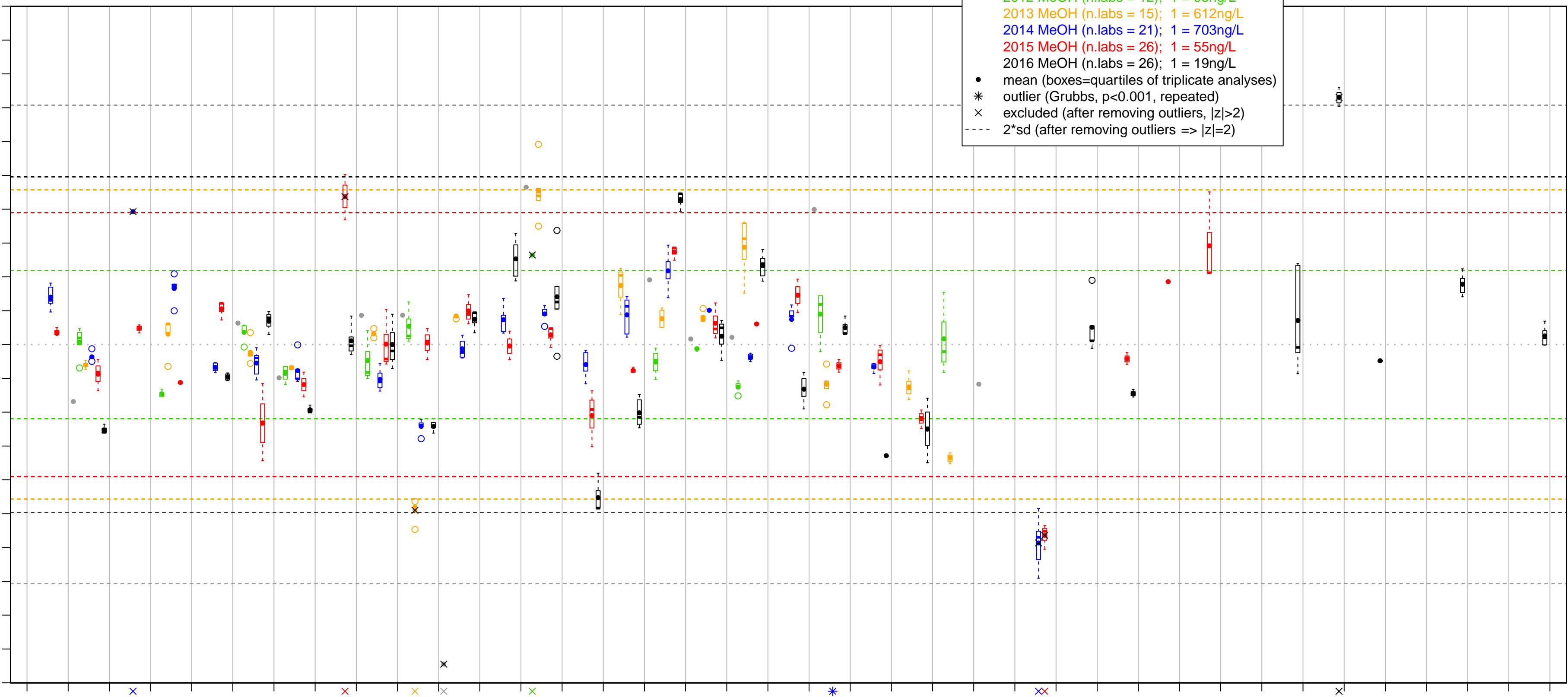
2.0
1.5
1.0
0.5
0.0

- 2011 MeOH (n.labs = 12); 1 = 507ng/L
- 2012 MeOH (n.labs = 12); 1 = 95ng/L
- 2013 MeOH (n.labs = 15); 1 = 612ng/L
- 2014 MeOH (n.labs = 21); 1 = 703ng/L
- 2015 MeOH (n.labs = 26); 1 = 55ng/L
- 2016 MeOH (n.labs = 26); 1 = 19ng/L
- mean (boxes=quartiles of triplicate analyses)
- * outlier (Grubbs, $p < 0.001$, repeated)
- x excluded (after removing outliers, $|z| > 2$)
- - - $2 \cdot sd$ (after removing outliers $\Rightarrow |z| = 2$)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37

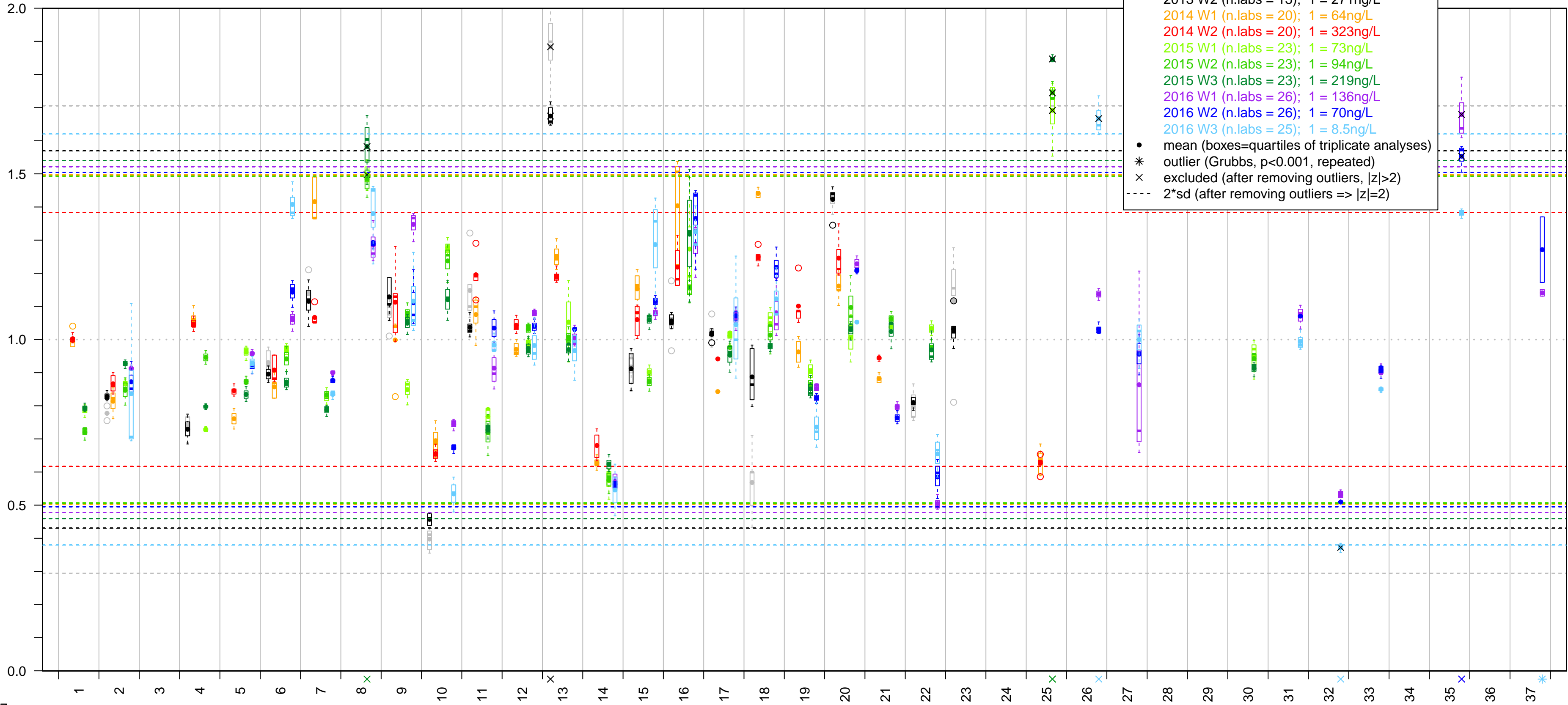
Lab ID

p16



MDMA Water

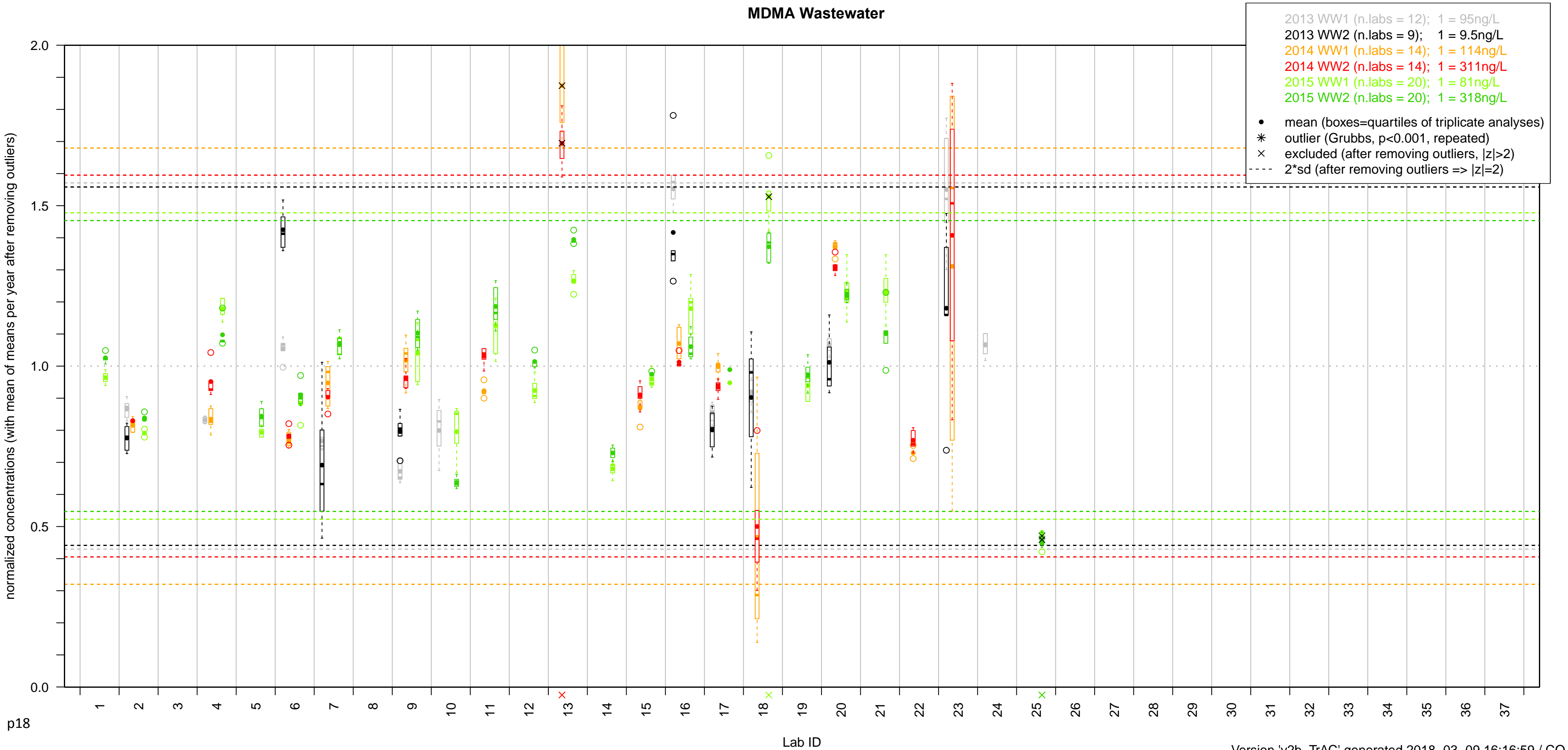
normalized concentrations (with mean of means per year after removing outliers)



p17

Lab ID

MDMA Wastewater



AMP MeOH

normalized concentrations (with mean of means per year after removing outliers)

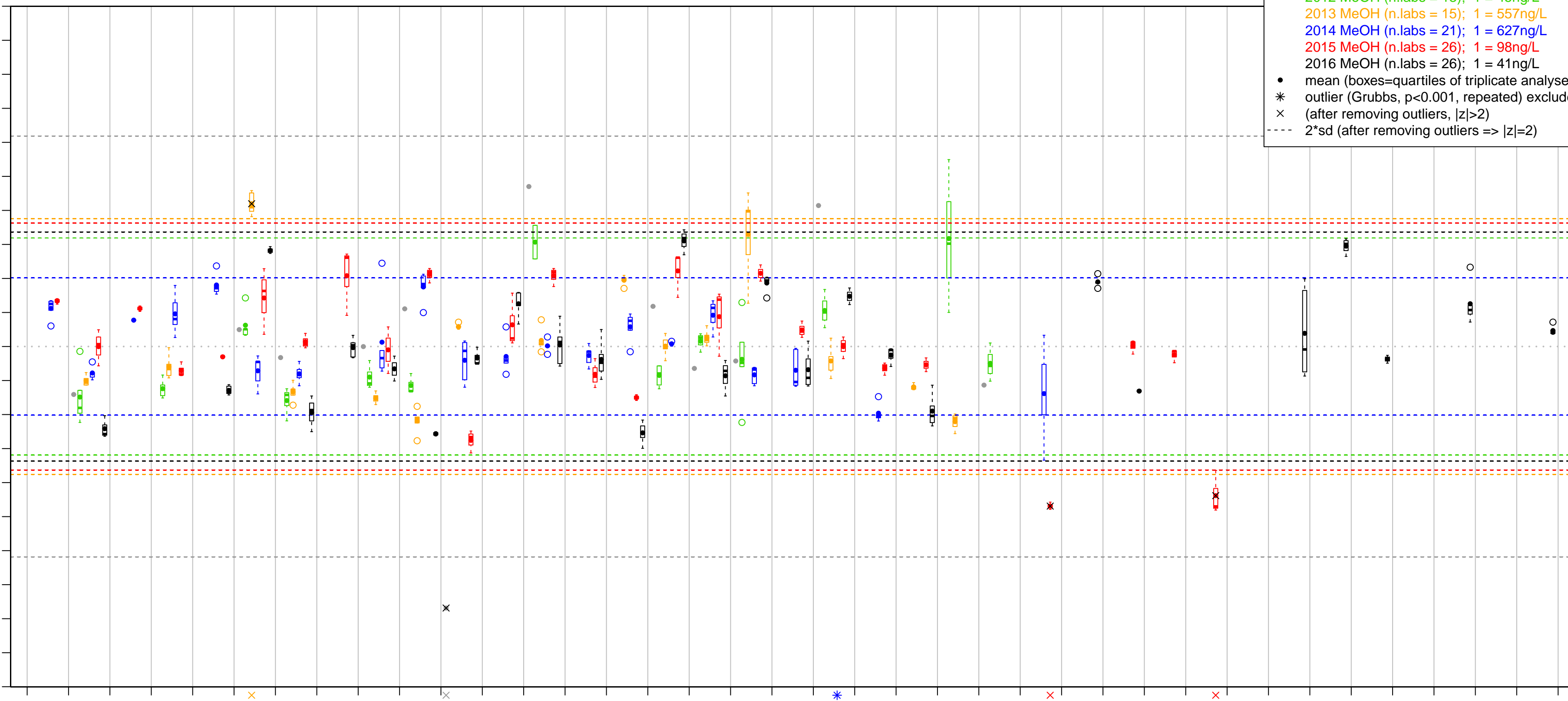
2.0
1.5
1.0
0.5
0.0

- 2011 MeOH (n.labs = 12); 1 = 513ng/L
- 2012 MeOH (n.labs = 13); 1 = 45ng/L
- 2013 MeOH (n.labs = 15); 1 = 557ng/L
- 2014 MeOH (n.labs = 21); 1 = 627ng/L
- 2015 MeOH (n.labs = 26); 1 = 98ng/L
- 2016 MeOH (n.labs = 26); 1 = 41ng/L
- mean (boxes=quartiles of triplicate analyses)
- * outlier (Grubbs, $p < 0.001$, repeated) excluded
- × (after removing outliers, $|z| > 2$)
- - - 2*sd (after removing outliers => $|z| = 2$)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37

Lab ID

p19



AMP Water

normalized concentrations (with mean of means per year after removing outliers)

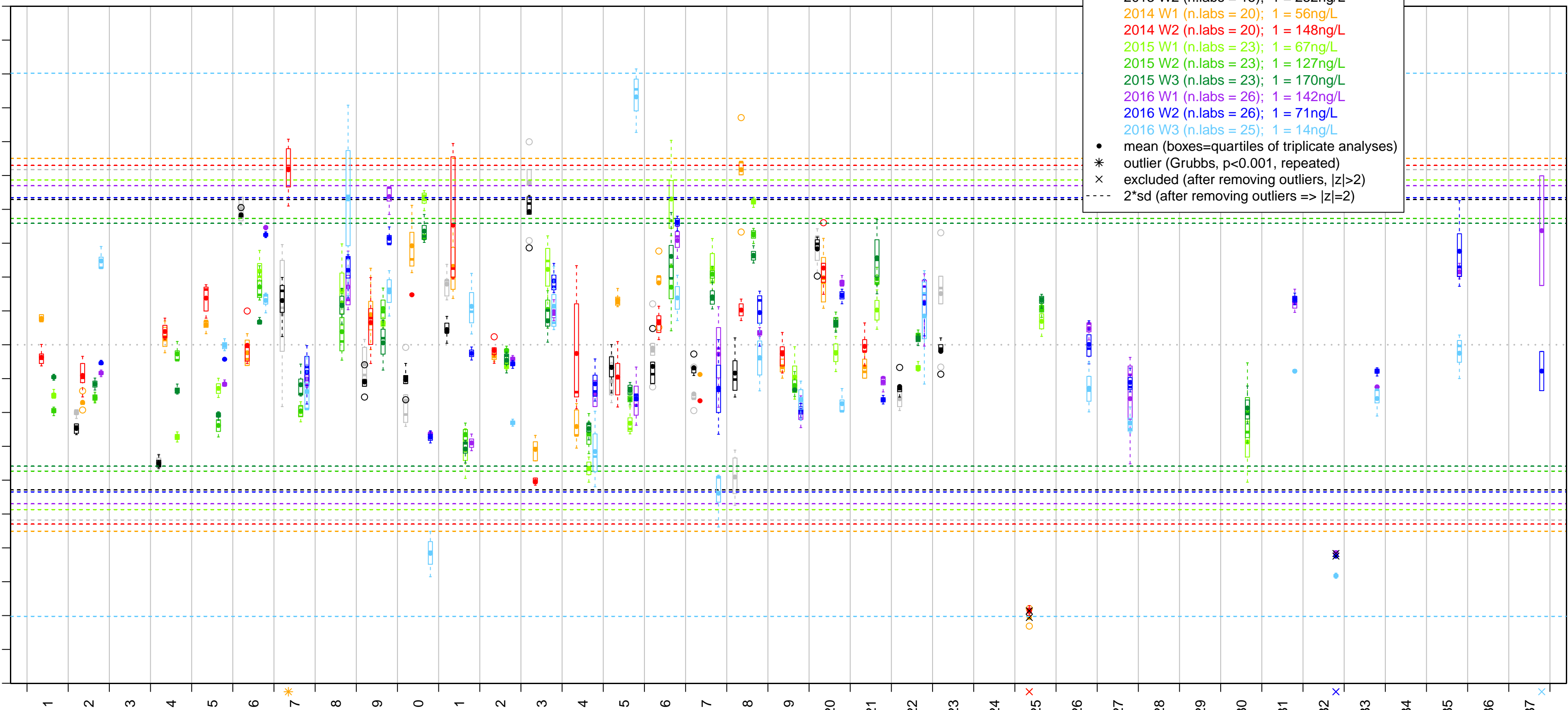
2.0
1.5
1.0
0.5
0.0

- 2013 W1 (n.labs = 15); 1 = 86ng/L
- 2013 W2 (n.labs = 15); 1 = 252ng/L
- 2014 W1 (n.labs = 20); 1 = 56ng/L
- 2014 W2 (n.labs = 20); 1 = 148ng/L
- 2015 W1 (n.labs = 23); 1 = 67ng/L
- 2015 W2 (n.labs = 23); 1 = 127ng/L
- 2015 W3 (n.labs = 23); 1 = 170ng/L
- 2016 W1 (n.labs = 26); 1 = 142ng/L
- 2016 W2 (n.labs = 26); 1 = 71ng/L
- 2016 W3 (n.labs = 25); 1 = 14ng/L
- mean (boxes=quartiles of triplicate analyses)
- * outlier (Grubbs, $p < 0.001$, repeated)
- × excluded (after removing outliers, $|z| > 2$)
- 2*sd (after removing outliers => $|z| = 2$)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37

Lab ID

p20

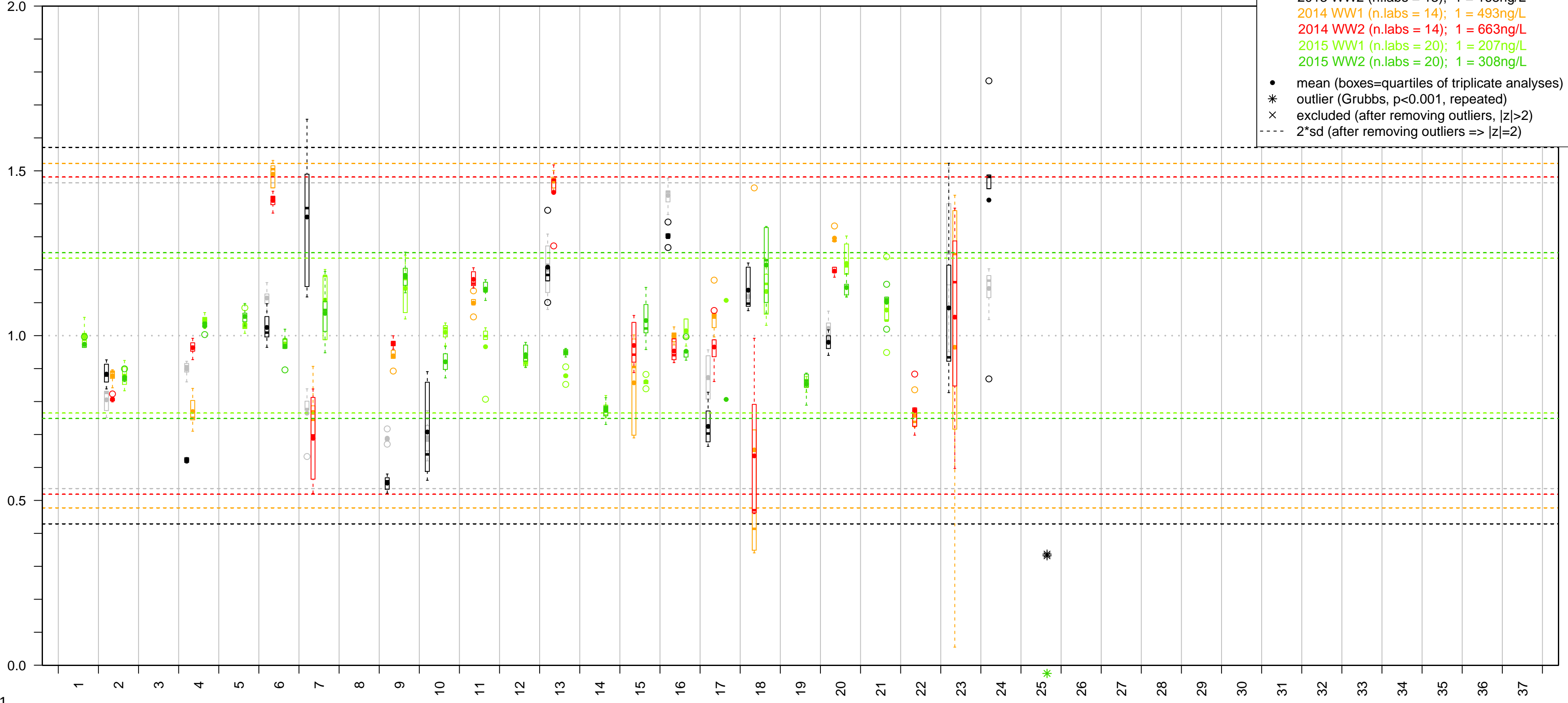


AMP Wastewater

normalized concentrations (with mean of means per year after removing outliers)

2013 WW1 (n.labs = 13); 1 = 389ng/L
 2013 WW2 (n.labs = 13); 1 = 168ng/L
 2014 WW1 (n.labs = 14); 1 = 493ng/L
 2014 WW2 (n.labs = 14); 1 = 663ng/L
 2015 WW1 (n.labs = 20); 1 = 207ng/L
 2015 WW2 (n.labs = 20); 1 = 308ng/L

- mean (boxes=quartiles of triplicate analyses)
- * outlier (Grubbs, $p < 0.001$, repeated)
- × excluded (after removing outliers, $|z| > 2$)
- 2*sd (after removing outliers => $|z| = 2$)



p21

Lab ID

METH MeOH

normalized concentrations (with mean of means per year after removing outliers)

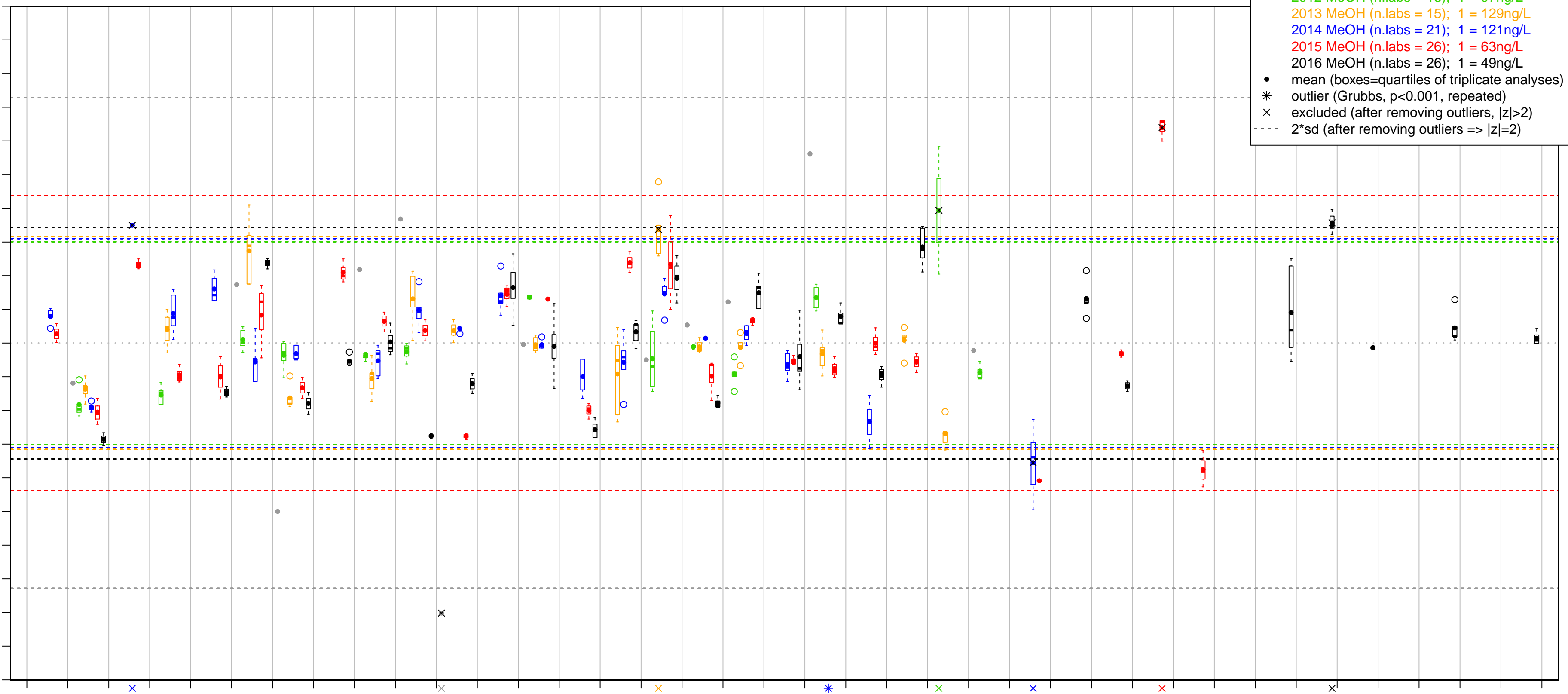
2.0
1.5
1.0
0.5
0.0

- 2011 MeOH (n.labs = 12); 1 = 457ng/L
- 2012 MeOH (n.labs = 13); 1 = 97ng/L
- 2013 MeOH (n.labs = 15); 1 = 129ng/L
- 2014 MeOH (n.labs = 21); 1 = 121ng/L
- 2015 MeOH (n.labs = 26); 1 = 63ng/L
- 2016 MeOH (n.labs = 26); 1 = 49ng/L
- mean (boxes=quartiles of triplicate analyses)
- * outlier (Grubbs, $p < 0.001$, repeated)
- x excluded (after removing outliers, $|z| > 2$)
- - - 2*sd (after removing outliers => $|z| = 2$)

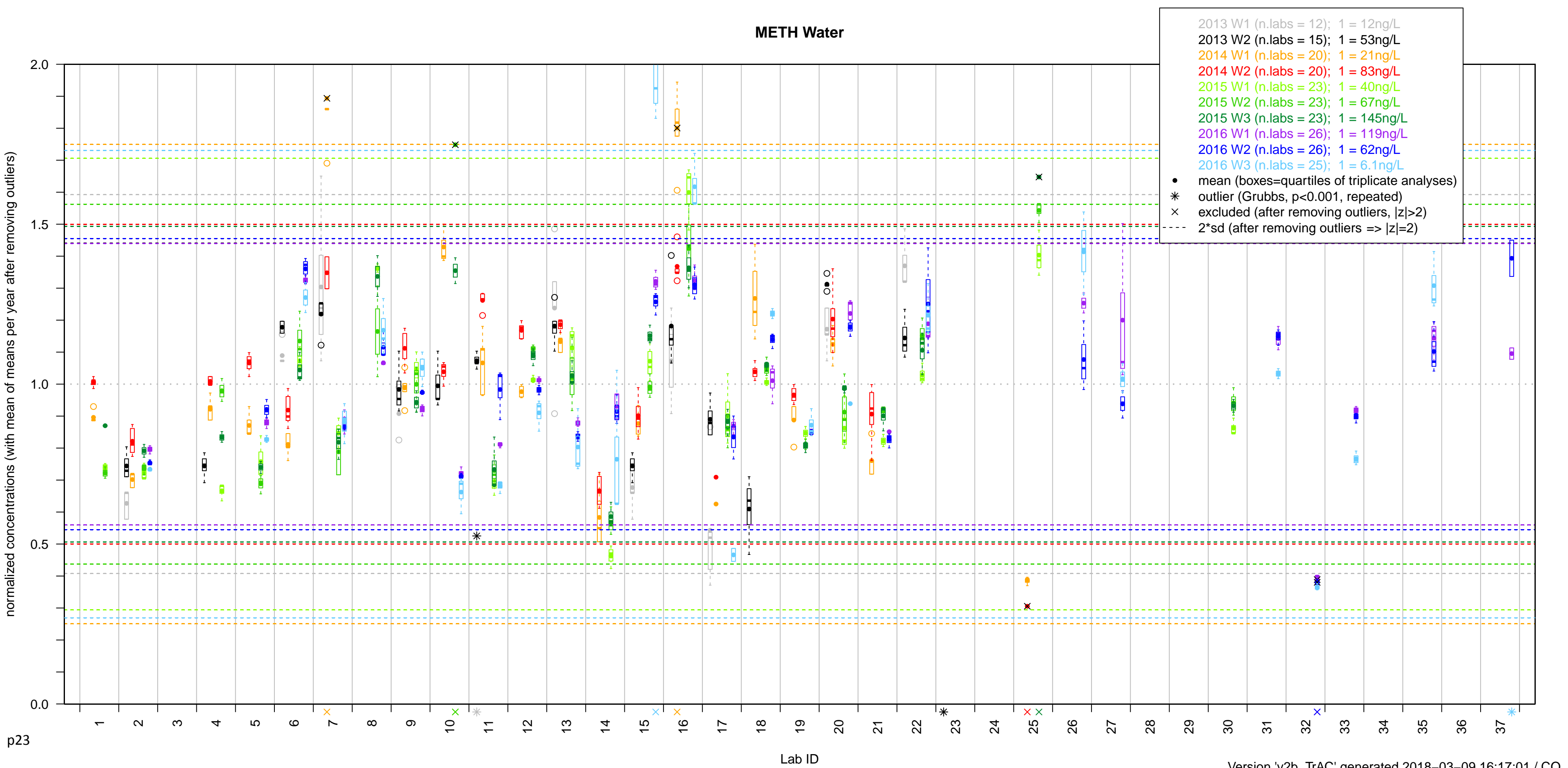
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37

Lab ID

p22



METH Water



METH Wastewater

normalized concentrations (with mean of means per year after removing outliers)

2.0
1.5
1.0
0.5
0.0

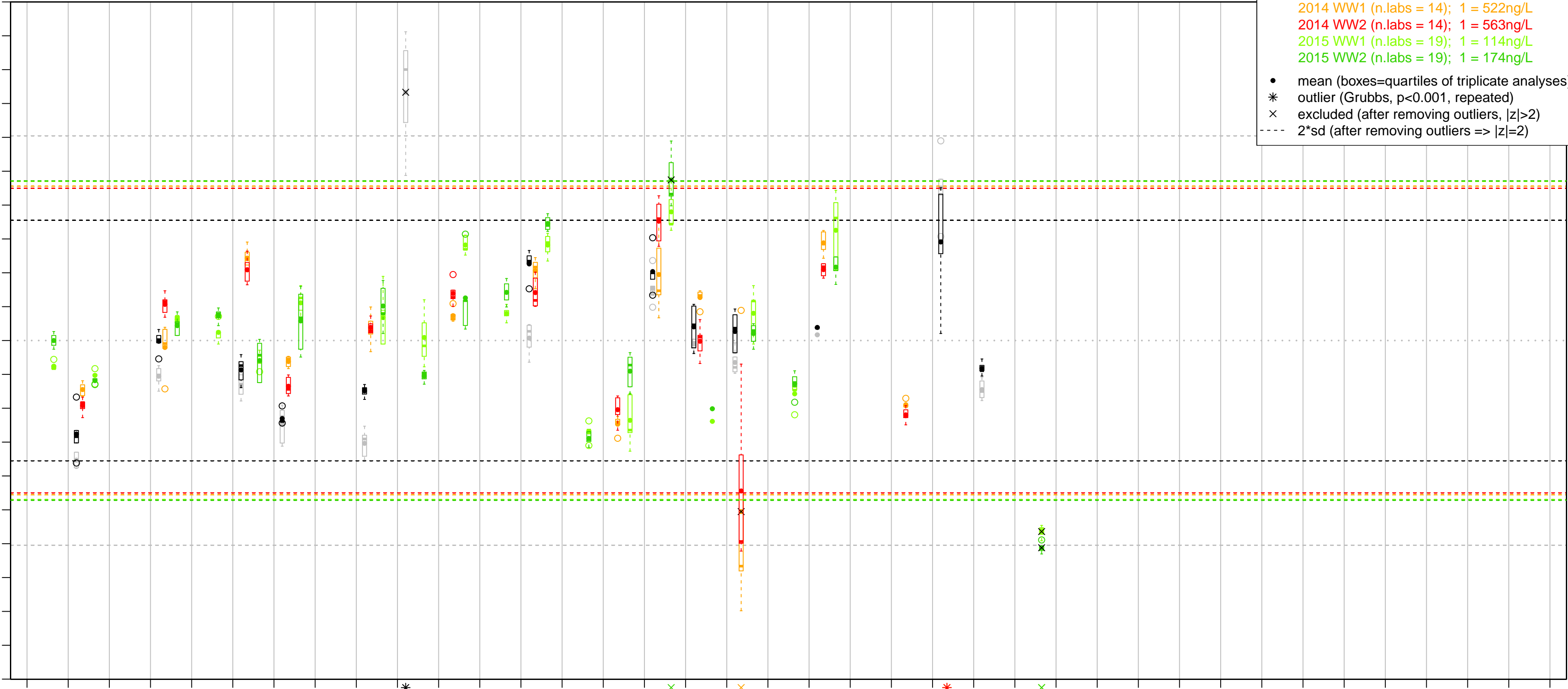
2013 WW1 (n.labs = 13); 1 = 422ng/L
2013 WW2 (n.labs = 13); 1 = 318ng/L
2014 WW1 (n.labs = 14); 1 = 522ng/L
2014 WW2 (n.labs = 14); 1 = 563ng/L
2015 WW1 (n.labs = 19); 1 = 114ng/L
2015 WW2 (n.labs = 19); 1 = 174ng/L

- mean (boxes=quartiles of triplicate analyses)
- * outlier (Grubbs, $p < 0.001$, repeated)
- × excluded (after removing outliers, $|z| > 2$)
- - - 2*sd (after removing outliers => $|z| = 2$)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37

Lab ID

p24



THC-COOH MeOH

normalized concentrations (with mean of means per year after removing outliers)

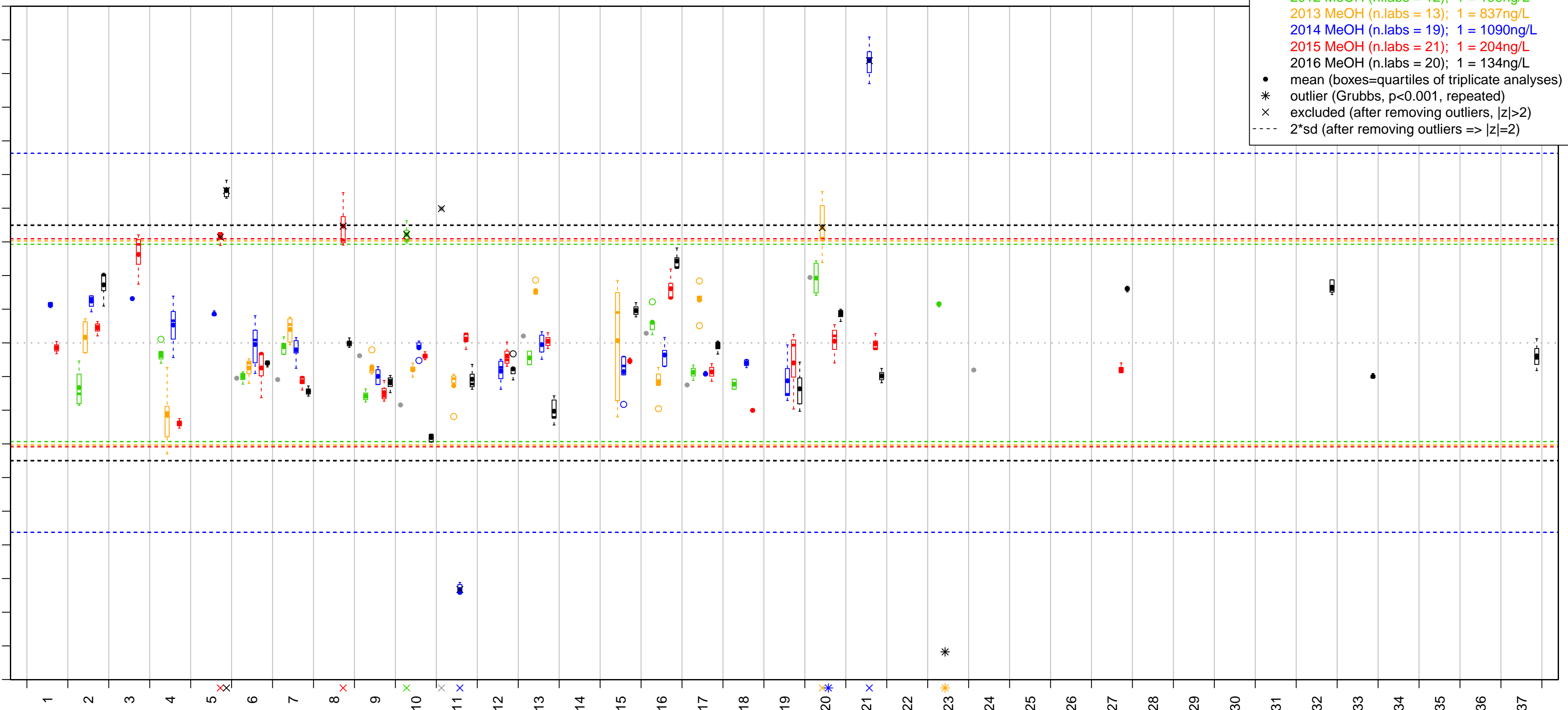
2.0
1.5
1.0
0.5
0.0

- 2011 MeOH (n.labs = 10); 1 = 525ng/L
- 2012 MeOH (n.labs = 12); 1 = 199ng/L
- 2013 MeOH (n.labs = 13); 1 = 837ng/L
- 2014 MeOH (n.labs = 19); 1 = 1090ng/L
- 2015 MeOH (n.labs = 21); 1 = 204ng/L
- 2016 MeOH (n.labs = 20); 1 = 134ng/L
- mean (boxes=quartiles of triplicate analyses)
- * outlier (Grubbs, $p < 0.001$, repeated)
- × excluded (after removing outliers, $|z| > 2$)
- - - 2*sd (after removing outliers $\Rightarrow |z| = 2$)

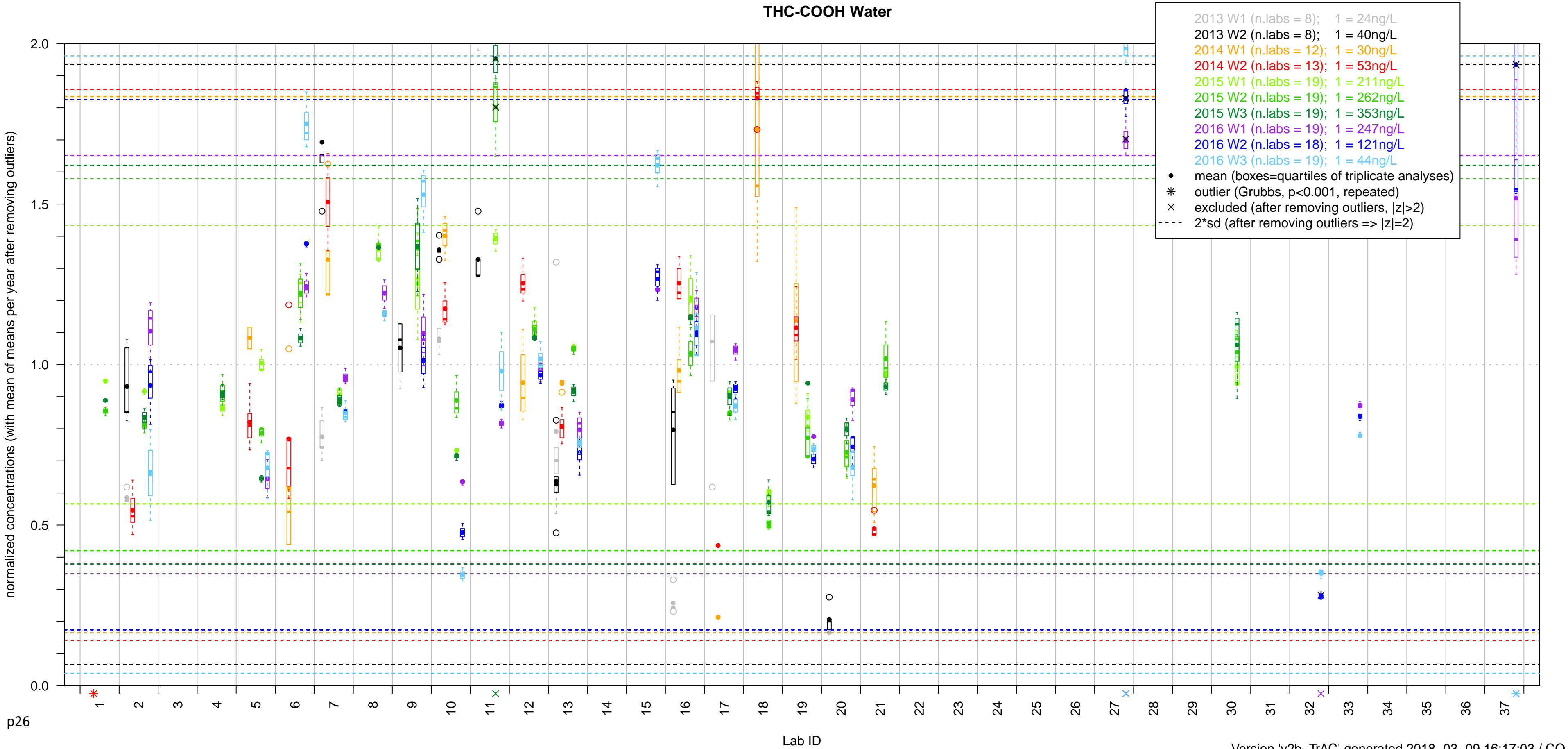
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37

Lab ID

p25



THC-COOH Water

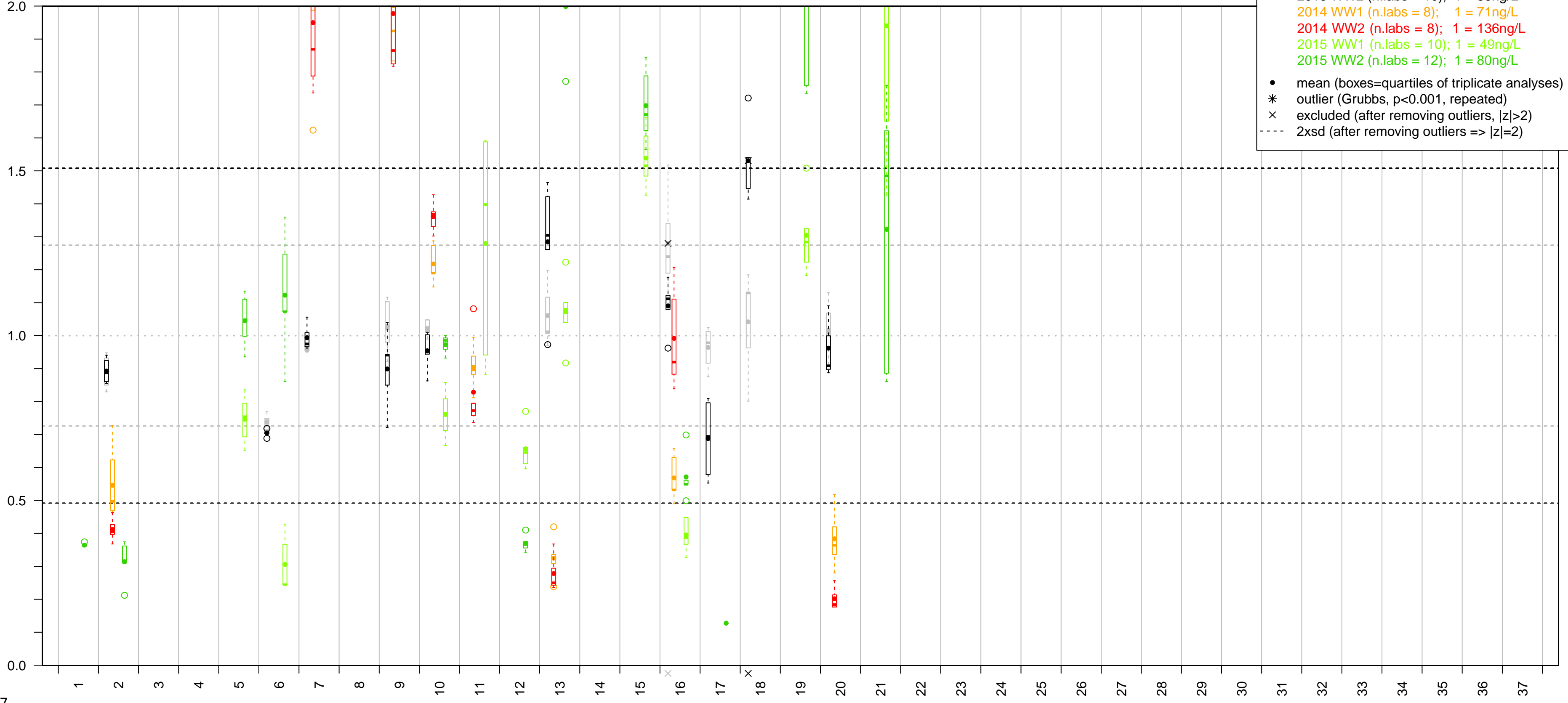


THC-COOH Wastewater

normalized concentrations (with mean of means per year after removing outliers)

2013 WW1 (n.labs = 10); 1 = 213ng/L
 2013 WW2 (n.labs = 10); 1 = 88ng/L
 2014 WW1 (n.labs = 8); 1 = 71ng/L
 2014 WW2 (n.labs = 8); 1 = 136ng/L
 2015 WW1 (n.labs = 10); 1 = 49ng/L
 2015 WW2 (n.labs = 12); 1 = 80ng/L

- mean (boxes=quartiles of triplicate analyses)
- * outlier (Grubbs, $p < 0.001$, repeated)
- × excluded (after removing outliers, $|z| > 2$)
- - - 2xstd (after removing outliers => $|z| = 2$)



p27

Lab ID

6-MAM MeOH

normalized concentrations (with mean of means per year after removing outliers)

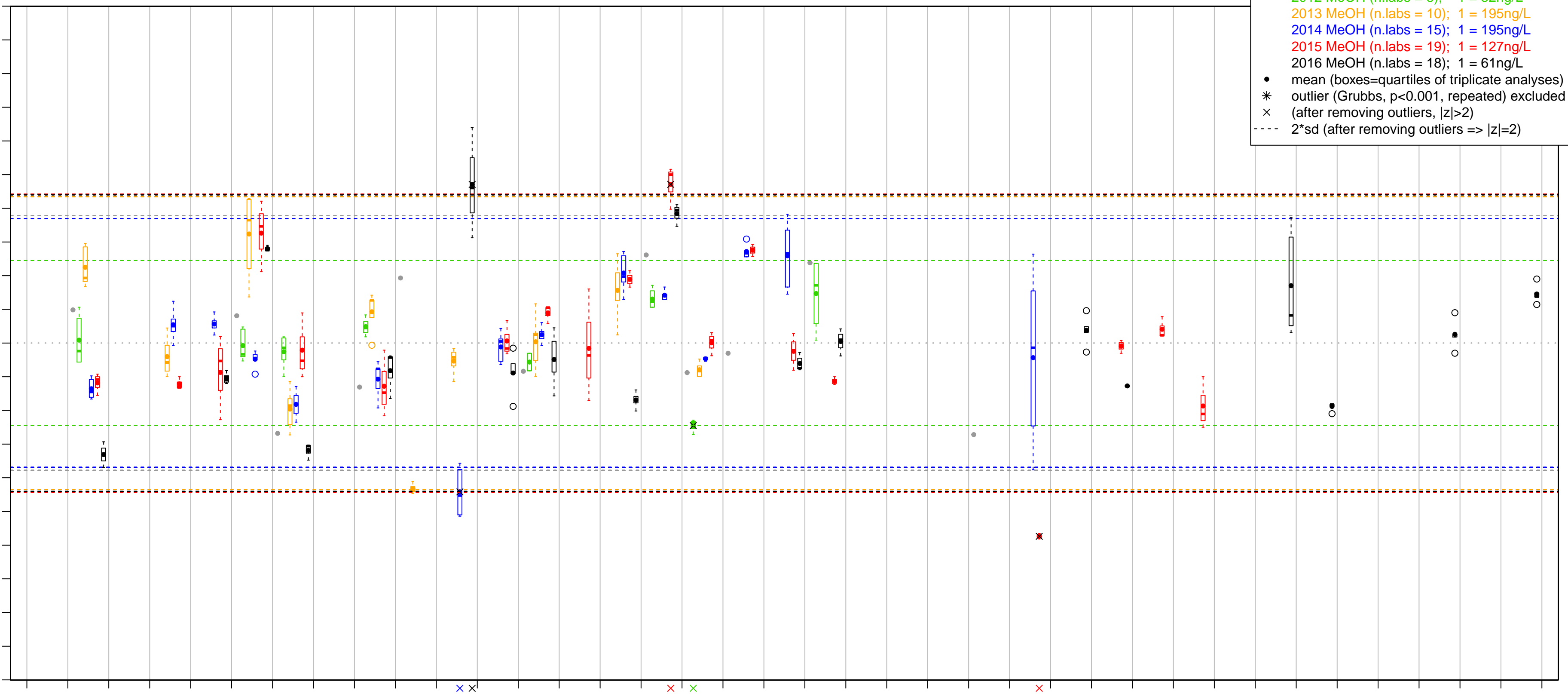
2.0
1.5
1.0
0.5
0.0

- 2011 MeOH (n.labs = 11); 1 = 472ng/L
- 2012 MeOH (n.labs = 8); 1 = 32ng/L
- 2013 MeOH (n.labs = 10); 1 = 195ng/L
- 2014 MeOH (n.labs = 15); 1 = 195ng/L
- 2015 MeOH (n.labs = 19); 1 = 127ng/L
- 2016 MeOH (n.labs = 18); 1 = 61ng/L
- mean (boxes=quartiles of triplicate analyses)
- * outlier (Grubbs, $p < 0.001$, repeated) excluded
- × (after removing outliers, $|z| > 2$)
- - - 2*sd (after removing outliers => $|z| = 2$)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37

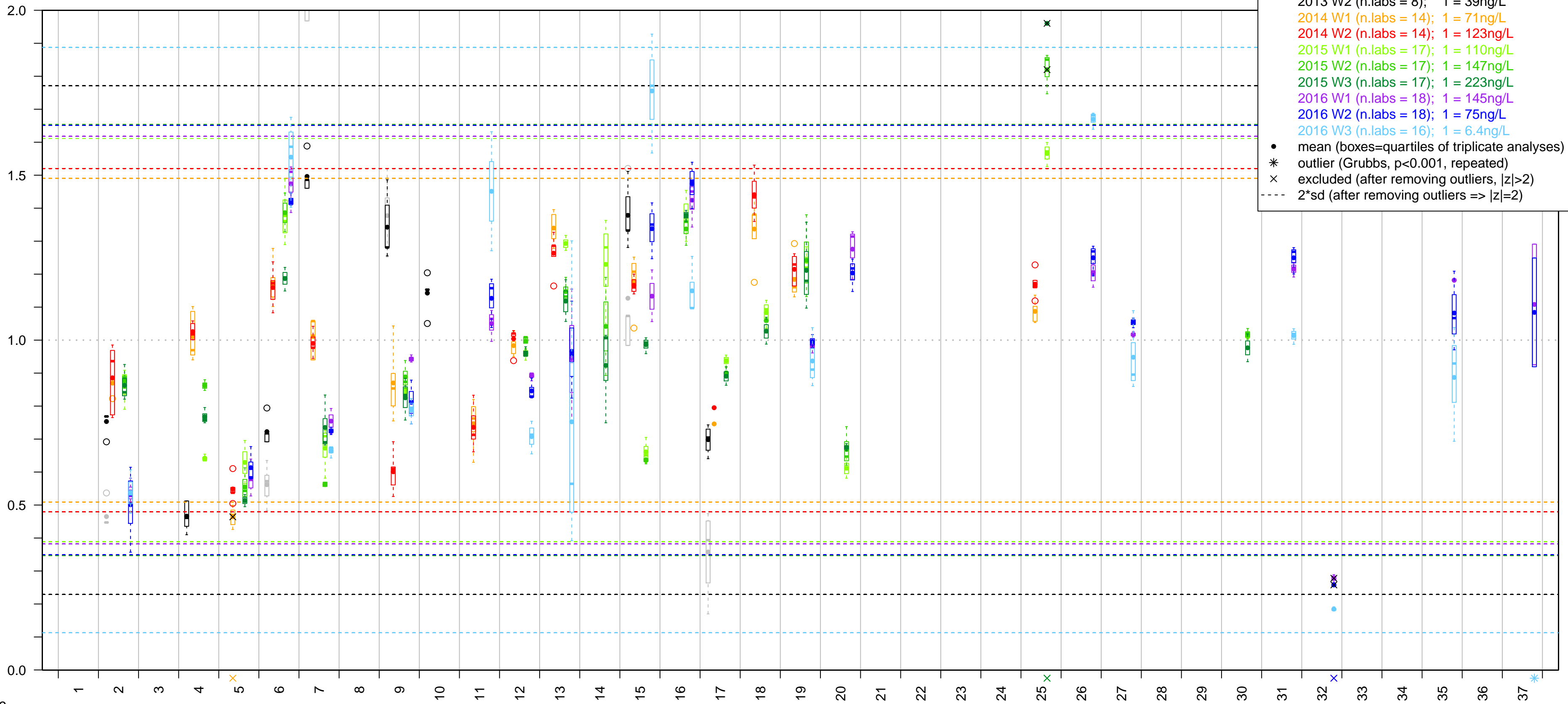
Lab ID

p28



6-MAM Water

normalized concentrations (with mean of means per year after removing outliers)

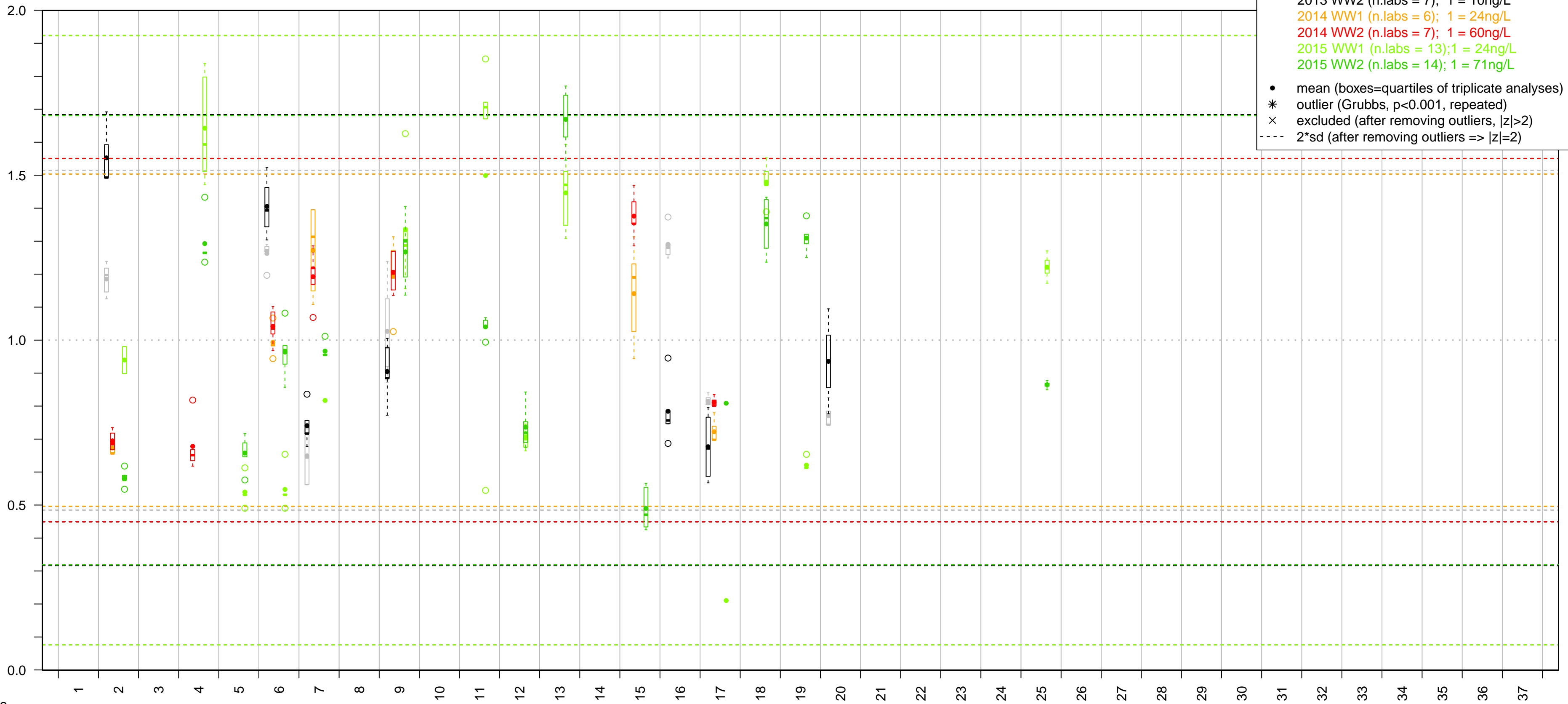


p29

Lab ID

6-MAM Wastewater

normalized concentrations (with mean of means per year after removing outliers)



p30

Lab ID