1   **Risk assessment for drugs of abuse in the Dutch watercycle**

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18 **ABSTRACT**

19   A screening campaign of drugs of abuse (DOA) and their relevant metabolites in the

20 aqueous environment was performed in the Netherlands. The presence of DOA, together with

21 the potential risks for the environment and the possible human exposure to these compounds

22 through consumption of drinking water was investigated. Sewage water (influent and

23 effluent), surface water of the rivers Rhine and Meuse, and drinking water (raw and finished)

24 were analysed by four different laboratories using fully in-house validated methods for a total

25 number of 34 DOA and metabolites. In this way, data reported for several compounds could

26 also be confirmed by other laboratories, giving extra confidence to the results obtained in this

27 study. 17 and 22 DOA were detected and quantified in influent and effluent sewage samples,

28 respectively. The tranquilizers oxazepam and temazepam, and cocaine and its metabolite

29 benzoylecgonine were found in high concentrations in sewage water. Nine compounds were

30 possibly not efficiently removed during treatment and were detected in surface waters. The

31 results indicated that substantial fractions of the total load of DOA and metabolites in the

32 rivers Rhine and Meuse enter the Netherlands from abroad. For some compounds, loads

33 appear to increase going downstream, which is caused by a contribution from Dutch sewage

34 water effluents. As far as data are available, no environmental effects are expected of the

35 measured DOA in surface waters.

36   In raw water, three DOA were detected, whereas in only one finished drinking water

37 out of the 17 tested, benzoylecgonine was identified, albeit at a concentration below the limit

38 of quantification (< 1 ng/L). Concentrations were well below the general signal value of 1

39 µg/L, which is specified for organic compounds of anthropogenic origin in the Dutch

40 Drinking Water Act.

41

43 Drugs of abuse, sewage water, surface water, drinking water, environmental risk

44 characterization

45

Ecological effects ?

Origin Drinking water
Groundwater 65%
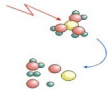Surface waters 35%

effluent

treatment

resource

Removal ?

influent

treatment

drinking water

Quality; safe ?

Removal ?

1. Introduction

Drugs of abuse (DOA) and their metabolites have recently been recognised as a novel group of environmental contaminants (Zuccato et al., 2008a). Owing to the increased sensitivity of analytical methods and the high level of world-wide consumption of DOA, they are among the growing number of emerging compounds that are detected at trace concentrations in the aqueous environment, including sewage water and surface waters.

DOA refers to both illegal drugs and misused prescription drugs, such as tranquilizers. They have received special attention recently since a novel approach allowed to study DOA consumption patterns of a population through sewage water analysis (Daughton, 2001; Zuccato et al., 2008b; van Nuijs et al., 2010; Thomas et al., 2012). Following consumption and excretion, some DOA and their metabolites are continuously released into the aquatic environment due to their insufficient elimination in sewage treatment plants (STPs) (Huerta-Fontela et al., 2008; Kasprzyk-Hordern et al., 2009; van Nuijs et al., 2009a; Postigo et al., 2010). Recent studies have shown the presence of DOA and their metabolites in STP effluents and river water in Australia (Irvine et al., 2011), Europe (Boleda et al., 2009; van Nuijs et al., 2009a; Postigo et al., 2010; Baker and Kasprzyk-Hordern, 2011; Hernandez et al., 2011) and North America (Jones-Lepp et al., 2004; Bartelt-Hunt et al., 2009).

Although the reported concentrations in surface waters are in general low, possible toxicological effects on animals, plants and humans may occur as a result of their presence in the aquatic environment. Especially, long-term effects on organisms and the effects of combined exposure to multiple compounds are of potential concern. However, so far, little ecotoxicological information for DOA is available and a well-founded scientific risk assessment is not yet possible. Although some information is available on DOA removal and transformation products formed during (drinking) water treatment processes (Huerta-Fontela et al., 2008), much more research is required for a better knowledge and understanding of

71 these processes. In the Netherlands, where approximately 40% of the drinking water is

72 produced from surface water, little is known about the occurrence of DOA and their

73 metabolites in the Dutch water cycle. Exploratory studies conducted in the period 2007-2010

74 have revealed the presence of benzoylecgonine, methadone, codeine and three tranquilizers

75 (nordazepam, temazepam and oxazepam) in Dutch surface waters and sewage effluents (de

76 Voogt et al., 2011; Hogenboom et al., 2009). The results from this study implied a clear need

77 for a more detailed monitoring campaign in the Netherlands.

78   This work presents the results of a large monitoring exercise on the occurrence of

79 DOA and metabolites in the Dutch watercycle. To the best of our knowledge, this study is one

80 of the largest of this kind in Europe, both in terms of number of analytes investigated and

81 types of water studied. In addition, samples were individually analysed by four different

82 laboratories, using their own validated analytical methodology. Five DOA were determined

83 by all four laboratories and additional seven by at least two laboratories. The fact that three

84 DOA (amphetamine, MDMA and benzoylecgonine) were found in several water samples by

85 all laboratories allowed the performance of an interlaboratory exercise.

86   Beforehand, a selection of compounds was made, applying the following criteria: the

87 results of the aforementioned preliminary inventory studies; international occurrence data on

88 DOA and metabolites in the aqueous environment (Baker and Kasprzyk-Hordern, 2011;

89 Bartelt-Hunt et al., 2009; Boleda et al., 2009; Hernandez et al., 2011; Irvine et al., 2011;

90 Jones-Lepp et al., 2004; Postigo et al., 2010; van Nuijs et al., 2009a); the estimated DOA

91 consumption in the Netherlands, which was based on criteria such as (il)legal import volumes

92 and anonymous surveys (van Laar et al., 2007), the availability of reference standards and

93 internal isotope-labelled standards, and the scope of the methods applied by the different

94 laboratories participating.

95   The main objectives pursued within this study were (1) to evaluate the occurrence of

96   DOA and metabolites in the Dutch watercycle (sewage influents and effluents, surface water

97   and drinking water); (2) to perform an ecotoxicological risk assessment of the levels of DOA

98   observed in surface waters.

99

2. Methods and materials

2.1. Sampling sites and sample collection

The sampling campaign in this study was performed by the Dutch National Institute for Public Health and the Environment (RIVM). All water samples were analysed by three laboratories: RIVM, KWR Watercycle Research Institute and University Jaume I (UJI). In addition, sewage water samples from four STPs (Utrecht, Apeldoorn, Amsterdam, Eindhoven) were also analysed by the University of Antwerp (UA).

Figure S1 of the Supplementary Information (SI) presents an overview of the sampling locations. Samples were collected from 65 sites and corresponded to three different types of water:

(1) Surface water: samples were collected at all nine surface water intake points for drinking water production in the Netherlands. Eight of these locations were part of the Meuse and Rhine river basins, and one was part of the Ems river basin. In addition, samples were taken at five locations along the rivers Rhine and Meuse.

(2) Raw water and finished drinking water: samples were taken at ten production sites where drinking water is produced from surface water and another seven drinking water production sites where drinking water is produced from river bank filtration. Raw water refers to the source water that enters the drinking water production facility. At some production sites this raw water has undergone pre-treatment, e.g., direct filtration, subsoil passage in the dune areas or storage in a reservoir, before it enters the drinking water treatment plant. Finished drinking water refers to water that is distributed as tap water. Drinking water treatment mostly consists of a combination of coagulation/flocculation and filtration/flotation, UV/H_2O_2 treatment or ozonation followed by activated carbon filtration.

125   (3) Sewage water: influent and effluent water samples were collected from eight STPs.

126 The size of these conventional biological treatment facilities varies from 37,000 to 1 million

127 equivalent-inhabitants.

128

129   Samples were collected in 2009 between October 4th and November 1st. At each

130 sampling location for surface and drinking water, grab samples were taken. At the drinking

131 water production sites, both raw water and finished drinking water were sampled on the same

132 day, without accounting for lag-time. At the STPs, 24-hour flow dependent samples from

133 influent and effluent were collected on the same weekend day, without accounting for lag-

134 time within the STP. All samples were collected in amber glass bottles, and transported and

135 stored in the dark at 4 °C.

136

137 *2.2. Selection of analytes*

138   A total of 34 DOA and metabolites belonging to 6 different chemical classes were

139 selected. The list of compounds, and isotopically labelled internal standards (ILIS) used for

140 matrix effects correction and quantification, by the four participating laboratories, and details

141 on preparation and storage of standard solutions are given in SI and **Table S1**.

142

143 *2.3. Analytical methods*

144   **Table 1** presents an overview of the main characteristics of the analytical methods

145 used by the four laboratories that participated in this study.

146   Sample clean-up and preconcentration was achieved by off-line solid-phase extraction

147 (SPE). Analyses of the final sample extracts were performed by liquid chromatography

148 coupled to tandem mass spectrometry (LC-MS/MS). All instruments employed electrospray

149 ionization (ESI) operating in positive mode. The applied mass spectrometric techniques were

150   triple quadrupole mass analyzers (QqQ), except for KWR that used high-resolution mass

151   spectrometry (LTQ FT Orbitrap). Further details on the analytical procedures and instrument

152   parameters can be found elsewhere (UJI (Bijlsma et al., 2009), KWR (de Voogt et al., 2011),

153   UA (van Nuijs et al., 2009b)), except for RIVM which is described in Supplementary

154   Information.

155

156   *2.4. Quality assurance*

157   The analytical methods used in the present study were validated in terms of linearity,

158   limit of detection (LOD), limit of quantification (LOQ), accuracy and precision (de Voogt et

159   al., 2011; Bijlsma et al., 2009; van Nuijs et al., 2009b). ILIS were used to compensate for

160   matrix effects (Hernández, 2005; Vanderford and Snyder, 2006). The identity of each of the

161   investigated analytes in samples of wastewater, surface water and drinking water was

162   confirmed by fulfilling relative retention time criteria and mass spectrometric identification

163   criteria (Commission Decision 2002/657/EC). An overview of the LOQs of the different

164   methods applied can be found in **Table S2**.

165

166   *2.5. Environmental risk characterization*

167   Environmental risk characterization for substances is usually performed by calculating

168   a Risk Characterization Ratio (RCR), which is a PEC/PNEC or MEC/PNEC ratio, in which

169   PNEC (Predicted No Effect Concentration) is an estimate for the highest concentration of

170   substance not affecting aquatic ecosystems, and PEC or MEC is the Predicted or Measured

171   Environmental Concentration in the aquatic environment. If the RCR is <1, no potential risk

172   to the aquatic environment is expected. A literature search was carried out to obtain PNECs

173   for the DOA detected in surface waters. In 2007, the Norwegian Pollution Control Authority

174   collected PNECs of pharmaceuticals, narcotics, and personal care products. For compounds

175   where no effect data were available, they used Quantitative Structure-Activity Relationship

176   (QSAR) or Ecological Structure Activity Relationships (ECOSAR) models to estimate the

177   potential effects of each compound (PNEC$_{ECOSAR}$) (Grung et al., 2007).

178

179   **3. RESULTS AND DISCUSSION**

180

181   *3.1. Comparative analysis between laboratories*

182   As mentioned above, all water samples were analysed by three laboratories: RIVM,

183   KWR and UJI. Some of the STP wastewater samples were also analysed by the UA. To the

184   best of our knowledge, this study is unique with respect to the number of different

185   laboratories and methodologies involved in analysing the same water samples. From the total

186   of 34 DOA and metabolites that were analysed in this monitoring campaign, 12 compounds

187   were analysed by two or more laboratories. Three of these DOA (amphetamine, MDMA, and

188   benzoylecgonine) were detected in sewage water by all four laboratories. This allowed us to

189   perform an extra validation of the methodology applied, a relevant aspect taking into account

190   the analytical difficulties associated with these complex sample matrices. So, in addition to

191   the criteria applied by each laboratory to assure quality, the deviations between the results

192   reported by the participants were used to prove the reliability of the analytical methods

193   applied.

194   **Table S3** shows comparative data obtained for the analysis of these three DOA in ten

195   sewage waters (analysed by four laboratories) and six surface waters (analysed by three

196   laboratories). Relative standard deviations (RSD) and overall average concentrations for the

197   16 samples analyzed are shown in the Supplementary Information. In general, the overall

198   (among laboratories) RSD was between 7 and 26%, with the exception of the RSD for

199   benzoylecgonine in two STP effluent samples (RSD = 38%). The fact that samples were

200 analyzed using different methodologies and that reported concentrations were comparable,

201 renders high confidence to the results obtained.

202

203 *3.2. Drugs of abuse and metabolites in the Dutch water cycle*

204 An overview of the monitoring results of DOA in the Dutch water cycle is presented

205 in **Table 2**. The average ± standard deviation (SD), range and median of the quantified levels

206 illustrate the dispersion and variation of the obtained results. Out of the total number of 34

207 DOA and metabolites analysed, 24 compounds were detected and quantified in sewage water,

208 9 in surface water, 3 in raw and none in finished drinking water. The presence of

209 benzoylecgonine was confirmed in one finished drinking water sample, but at a concentration

210 below the LOQ for this analyte (1 ng/L). It must be considered that only a single, 24-h

211 composite sample from the effluents was collected to estimate loads of DOA discharged from

212 the STP, and that these samples were collected during the weekend. It is well-known that

213 concentrations of some DOA are higher during the weekend compared to weekdays (Thomas

214 et al., 2012). So the average loads might be different from the loads calculated in this paper.

215 Therefore, this might be seen as the worst-case scenario because of the higher concentrations

216 found in sewage water. Similarly, loads of DOA into the rivers were calculated using only a

217 single grab sample per location, which is a limitation when comparing the loads from

218 different locations and countries. However, the data presented in this work provides a

219 valuable indication of the importance of STP discharges of DOA into the environment. The

220 daily and seasonal variations of discharge loads were not an objective of the present study and

221 should be evaluated in a new set of experiments.

222

223 *3.2.1. Occurrence in sewage water*

224   In STP influents, 17 compounds could be quantified, while for effluents 22

225   compounds showed concentrations > LOQ (**Table 2**).   The compounds found in the STP

226   influents were also detected in the STP effluents, except for THC-COOH and cocaethylene,

227   whereas MDA, diazepam, nordazepam, fentanyl, ketamine, methcathinone and ritalin were

228   solely found in effluents. Deconjugation within the STP, transformation of compounds (e.g. in

229   the case of benzodiazepines), the higher LOQs in influent samples compared with effluents,

230   or a combination of these processes might explain the exclusive presence and/or higher

231   concentrations found in effluent compared to influent samples (Bones et al., 2007; Kvanli et

232   al., 2008). To define which process occurs for which compound is beyond the scope of this

233   study and should be a focus of completely new experiments. Moreover, conclusions about

234   removal efficiencies of the STPs cannot be drawn based on this research, since STP influents

235   and effluents were collected on the same day and as a result lag-times were not taken into

236   account. In a later study, removal efficiencies and daily variations were investigated in an

237   extensive one week monitoring of 24 DOA and metabolites in Dutch influent and effluent

238   sewage water (Bijlsma et al., 2012). Occurrence of DOA monitored by both studies is in a

239   good agreement. From the 18 common compounds included in both studies, 14 compounds

240   were detected in influents and/or effluents in both cases. The only exceptions were MDA,

241   diazepam, morphine and fentanyl that were not found in any sewage water sample analyzed

242   by Bijlsma et al. (2012). In addition, nordazepam, ketamine and ritalin were mainly found in

243   effluents, which is in correspondence with the results of the present work. A preliminary

244   conclusion that can be drawn from the present study is that 22 out of 34 DOA were not

245   completely removed during sewage water treatment. As a consequence, substantial loads of

246   DOA and metabolites may enter receiving surface waters through STP effluents.

247   **Figure 1** shows the calculated loads of DOA discharged from the eight Dutch STP

248   effluents collected during a weekend day. The Amsterdam STP shows highest loads towards

249   surface water, up to 105 g/day of oxazepam. This can be related to the highest Inhabitant

250   Equivalent (I.E.) for this STP, and also to the higher consumption of DOA that is expected in

251   more urbanized areas or large cities (van Nuijs et al., 2009a; Banta-Green et al., 2009). Hence,

252   if removal efficiencies (%) are of the same order of magnitude for all STPs, higher discharges

253   can be expected when higher I.E.s are involved. However there are some exceptions,

254   indicating that other factors also play a role (e.g. consumption of certain DOA can be

255   regionally and temporally dependent). A noticeable discharge is shown for MDMA in

256   Amsterdam (up to 80 g/day, 10 fold more than any of the other STPs). An estimation of the

257   discharges expressed per inhabitant also indicated highest loads of MDMA for Amsterdam

258   (data not shown). In general, discharge values of DOA expressed per inhabitant correspond

259   when comparing the different cities. A possible explanation for the relative high MDMA

260   loads in Amsterdam could be the presence of an extensive club scene in this STP region. This

261   can be linked with a higher consumption of this 'party' drug. It is noteworthy that on the day

262   before sampling, a big Halloween dance party was celebrated. Due to the travel distance of

263   the sewer and the lag-time of the STP (24 h), sampling of the influent and effluent started

264   when the main discharge of this party was already under treatment in the STP. In the same

265   line, Bijlsma et al. (2009) reported high drug levels in sewage water samples due to a special

266   music event, and suggested that these high drug levels led to a decrease in the removal

267   efficiency.

268

269   *3.2.2. Occurrence in surface waters*

270   In the surface waters of the rivers Rhine and Meuse, 9 DOA were detected (**Table 2**).

271   Oxazepam, temazepam and benzoylecgonine were most abundantly present (in > 70% of the

272   sampling locations) and concentrations were highest for the benzodiazepines, with a

273   maximum value of 68 ng/L for oxazepam. These findings are consistent with relatively high

274   levels of benzodiazepines observed in influents and the relatively poor removal rate in Dutch

275   STPs (Bijlsma et al., 2012). Oxazepam and temazepam were among the top 10 most

276   prescribed pharmaceuticals in the Netherlands in 2008 (SFK, 2008). Other widely used

277   pharmaceuticals, such as various antibiotics, beta-blockers, lipid regulators or anti-

278   inflammatory pharmaceuticals were reported in comparable concentrations in the river Rhine

279   (ter Laak et al., 2010). In general, the levels of DOA and metabolites found in the river Meuse

280   were higher than those of the river Rhine, as shown in **Figure 2**, most probably as a result of

281   the larger dilution in the river Rhine which has a much larger flow rate than the river Meuse.

282   Based on our data, loads of DOA and metabolites through the Rhine and Meuse rivers can be

283   estimated. However it is worth mentioning that such estimations should be interpreted as

284   indicative since they are based on grab samples and on a single sampling date.

285   The loads of DOA and metabolites transported by rivers are calculated by multiplying

286   the concentrations (ng/L) with the flow rate (L/day) recorded at the sample location on the

287   sampling date. Flow rates on the sampling dates were obtained from the Dutch Ministry of

288   Waterworks database. Higher flow rates in the river Rhine led to higher estimated loads in

289   this stream (**Figure 3** and **Table S4)**. Loads were also calculated at two locations

290   downstream: Keizersveer (river Meuse) and Maassluis (river Rhine). As shown in **Figure 3**

291   and **Tables S4** and **S5**, the loads generally increased downstream for the four compounds

292   presented. An increase of the riverine loads during passage of the rivers Rhine and Meuse

293   through the Netherlands is plausible, because oxazepam, temazepam and codeine are

294   consumed in the Netherlands in quantities of approximately 200 - 1500 kg per year, according

295   to sales data from the Foundation for Pharmaceutical Statistics in the Netherlands (SFK,

296   2008). **Table S4** shows that for the river Rhine, the increase in loads downstream along the

297   Dutch part of the river is of the same order of magnitude as the contribution from abroad for

298   temazepam and oxazepam, whereas for benzoylecgonine and codeine the contribution from

299   abroad is larger. For the river Meuse, the increase in loads for temazepam and oxazepam

300   downstream along the Dutch part of the river seems higher than the contribution from abroad

301   (**Table S5**). However, for the river Meuse there may also be a contribution from Belgian and

302   German tributaries that discharge their waters into the river Meuse downstream from Eijsden.

303   For benzoylecgonine and codeine loads are even decreasing downstream along the Dutch part

304   of the river, which cannot be explained. Although these calculations are only indicative with

305   considerable uncertainties, they imply that, when mitigation measures like for example

306   improved sewage treatment are considered, these should be implemented both in Dutch and in

307   Belgian / German STPs in order to effectively lower concentrations of DOA in Rhine and

308   Meuse rivers. However, more data is needed to draw definite conclusions on this matter.

309   An attempt was made to compare the increase in loads downstream along the Dutch

310   part of the rivers Rhine and Meuse with the loads from Dutch inhabitants in the Rhine and

311   Meuse catchment. Bijlsma et al (2012) showed that considerable levels of these compounds

312   can reach the Dutch surface waters through STP effluent discharges since they are not

313   efficiently removed in STPs. This potential contribution from Dutch inhabitants was

314   estimated based on the average DOA loads from the 8 STPs per I.E. discharged to surface

315   water, multiplied with the total amount of Dutch inhabitants in Rhine (ICBR, 2009) and

316   Meuse (IMC, 2008) catchments, respectively. The calculated loads are shown in **Table S4**

317   and **Table S5**. The increase in loads at the downstream stations Keizersveer (Meuse) and

318   Maassluis (Rhine) should be comparable to the estimated loads from the Dutch STPs if

319   degradation in the environment would not occur. **Table S4** and **Table S5** however show that

320   the loads from STPs are about an order of magnitude larger than the increase in loads at the

321   downstream stations. This means that, despite the high insecurity of the calculations which is

322   shown by the high standard deviations, also degradation in the environment might play a role.

323

3.2.3. Occurrence in the drinking water production chain

325 **Figure 4** presents average concentrations of DOA and metabolites observed during

326 several stages of the drinking water production chain. Samples (from water intake locations,

327 raw water and finished drinking water) were collected from three types of production

328 processes where drinking water is prepared from surface waters (direct treatment and with

329 soil aquifer recharge), and from bank filtrate. It has to be stressed here that the monitoring

330 results are not entirely suitable to evaluate the effectiveness of the different treatment steps,

331 since both the raw waters and finished drinking waters were sampled only once, on the same

332 day and without accounting for lag-times. The results should therefore be regarded as

333 indicative, and are used here merely to provide a visualisation and qualitative assessment of

334 compounds that are not removed completely during drinking water treatment.

335 As shown in **Figure 4**, amphetamine-type stimulants, cocaine and its metabolites,

336 benzodiazepines and opiates are present in river water at the water intake locations. However,

337 in raw water only oxazepam, temazepam and benzoylecgonine were found, and at lower

338 concentrations. Apparently, these compounds are removed to some extent during the

339 treatment of raw water which includes direct filtration, subsoil passage in the dune areas or

340 storage in a reservoir. It takes place before the water enters the drinking water treatment plant

341 where further, more advanced treatment processes are used. Oxazepam and temazepam were

342 not detected in the raw water that is produced from bank filtrate: possibly they were removed

343 during bank filtration.

344 The treatment to produce finished drinking water mostly consists of a combination of

345 coagulation/flocculation and filtration/flotation, UV/H_2O_2 treatment or ozonation followed by

346 activated carbon filtration. It seemed effective in the removal of the compounds selected as

347 none of the DOAs investigated was detected, with the exception of benzoylecgonine that was

348 confirmed at a level between LOD and LOQ (< 1 ng/L) in a single finished drinking water.

349 Although in our study no DOA were detected in finished drinking water, Huerta-Fontela et al.

350 (2008) did detect benzoylecgonine in Spanish drinking water. In their study on the removal

351 efficiency of Spanish drinking water treatment plants, they concluded that benzoylecgonine

352 was still detected in most finished drinking waters at mean concentrations of 45 ng/L, even

353 though reductions of 90% were obtained during treatment which consists of prechlorination,

354 flocculation and sand filtration steps. Probably the use of rather advanced drinking water

355 treatment techniques in the Netherlands, like UV or ozonation, followed by activated carbon

356 filtration is more effective in reducing DOA.

357

358 *3.3. Environmental risk characterization*

359 The environmental risk characterization ratios were calculated by dividing the

360 maximum concentrations measured in surface water (MEC) by the reported PNEC or

361 $PNEC_{ECOSAR}$.

362 For oxazepam a PNEC was reported, and for codeine, cocaine, morphine, MDMA and

363 methamphetamine QSAR derived $PNEC_{ECOSAR}$ were available (Grung et al., 2007). For

364 temazepam and benzoylecgonine, no PNECs could be found in public literature. For

365 temazepam however, conforming to what was done for diazepam by Grung et al. (2007), the

366 PNEC for oxazepam was used as the default PNEC, as temazepam is also a benzodiazepine,

367 having a similar metabolic pathway as diazepam. For benzoylecgonine, the PNEC for cocaine

368 was used. Animal studies showed that benzoylecgonine is less toxic than cocaine, so the

369 PNEC for cocaine will be safe for benzoylecgonine as well. For methadone no PNECs could

370 be found or derived.

371 **Table 3** shows the calculated MEC/PNEC ratios, which are well below 1 (range:

372 0.0002 to 0.38), meaning that, as far as data are available, no environmental effects are

373 expected of the measured individual DOA in the surface water. However, most PNECs are

374   derived by ECOSAR modelling and it is questionable if this is the most appropriate model.

375   ECOSAR modelling provides acute PNEC$_{ECOSAR}$ data but with a very high degree of

376   uncertainty. The question is whether traditional approaches to extrapolating chronic PNECs

377   are at all relevant when considering narcotic substances. The acute/chronic ratio approach

378   which was applied is founded on the toxic mechanism of non-specific narcosis, which is by

379   definition not applicable to narcotics, which have a very specific effect. A high degree of

380   uncertainty is therefore associated with the modelled acute PNEC and any assumptions made

381   in terms of extrapolating chronic PNEC data (Grung et. al. 2007). Unfortunately, no published

382   aquatic ecotoxicological data for narcotic substances are available.

383

384   *3.4. Toxicological relevance for human health through drinking water*

385   In one finished drinking water sample benzoylecgonine was detected, but at a

386   concentration below the LOQ for this analyte (1 ng/L). Detection of this cocaine metabolite

387   has also been reported in Spanish drinking water although at higher concentrations, with a

388   mean value of 45 ng/L (Huerts-Fontela et al. 2008). No other DOA were found to be present

389   in finished water, therefore no human health risks are expected.

390   Currently, for individual DOA no statutory drinking water guideline values are

391   available from e.g. European Commission, US Environmental Protection Agency (EPA) or

392   World Health Organization (WHO). According to the Dutch Drinking Water Act a general

393   signal value of 1 µg/L applies to organic compounds of anthropogenic origin for which no

394   individual statutory drinking water guidelines are specified. For the twelve DOA that were

395   detected in surface water and the five DOA that were detected in raw (process) water, the

396   concentrations were well below this signal value. Although more research and data are

397   needed, the results from this study suggest that the presence of DOA in drinking water should

398   not be a cause of significant concern for human health.

399   .

4. CONCLUSIONS

400

401   This extensive screening campaign confirms the presence of DOA and metabolites at

402   low concentration levels in the Dutch water cycle. All samples were analysed by at least three

403   laboratories using different methodologies, a relevant and unique aspect in this type of work.

404   DOA and metabolites were detected and quantified in sewage water influents (17) and

405   effluents (22), surface water (9), and raw water (3). In finished drinking water only

406   benzoylecgonine was detected in one sample, but at a concentration below the LOQ for this

407   analyte (1 ng/L). No other DOA were found to be present in finished drinking water; therefore

408   no human health risks are expected. Concentrations of DOA observed in surface water and

409   raw water are well below the general signal value of 1 μg/L, which is specified for organic

410   compounds of anthropogenic origin in the Dutch Drinking Water Act. For DOA for which an

411   evaluation could be made, no environmental effects are expected of the measured

412   concentrations in surface water. However further research with respect to possible long-term

413   (chronic) effects on organisms and possible effects of combined exposure to multiple

414   compounds at low concentrations is recommended, and the development of analytical

415   techniques to detect possible new emerging DOA needs further attention.

416

426

427 **SUPPLEMENTARY INFORMATION**

428   In this section, useful information on the chemical and materials and the analytical

429 procedure used by RIVM are given. Additionally, an overview of the sampling locations is

430 given (Figure S1). Furthermore five tables are added: Table S1 provides the list of DOA

431 investigated by the four participating laboratories, Table S2 shows an overview of the LOQs

432 (ng/L) per compound, sample matrix and laboratory, Table S3 shows a comparison of results

433 obtained by different laboratories, Table S4 compares the estimated loads entering the river

434 Rhine through German STPs and through Dutch STPs, and Table S5 compares the estimated

435 loads entering the river Meuse through Belgian STPs and through Dutch STPs.

436

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FIGURE CAPTION

Figure 1. Estimated discharges (g/day) of DOA from STPs based on monitoring data and STP effluent flow rates in October 2009.
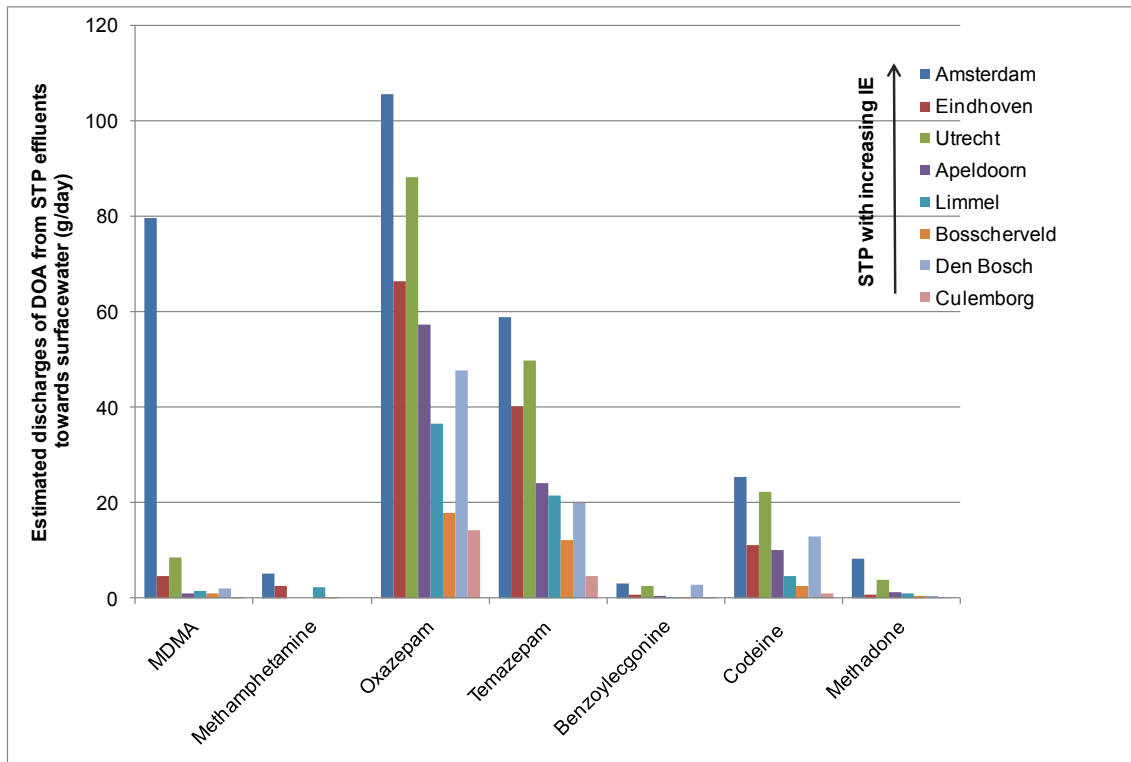
FIGURE CAPTION

Figure 2. Concentrations (ng/L) of DOA at border crossing locations (river Rhine: Lobith and river Meuse: Eijsden) and downstream (river Rhine at Maassluis and river Meuse at Keizersveer).
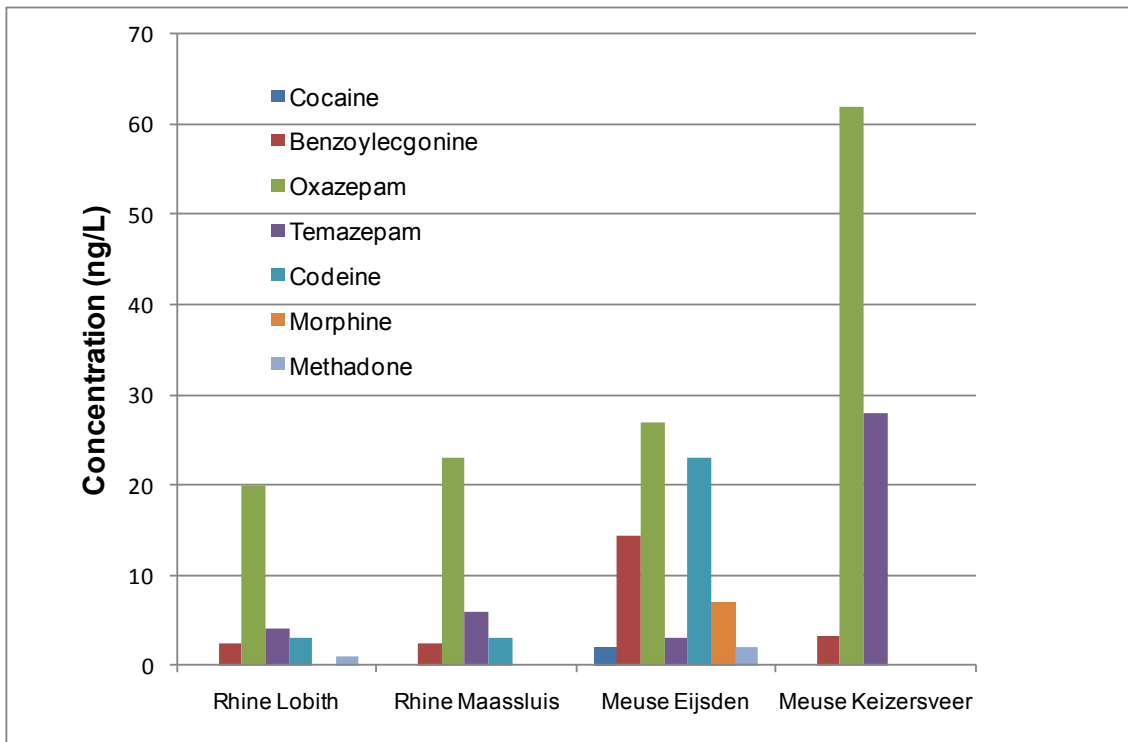
Figure 3. Estimated loads (g/day) of DOA in rivers Rhine and Meuse at Dutch border crossing locations (Lobith and Eijsden, respectively) and downstream (Maassluis and Keizersveer, respectively) calculated from monitoring data and river flow rates on one sampling date in October 2009.

Figure 4. Average concentrations (ng/L ± SD) of DOA in water collected from different stages of three types of drinking water production processes.
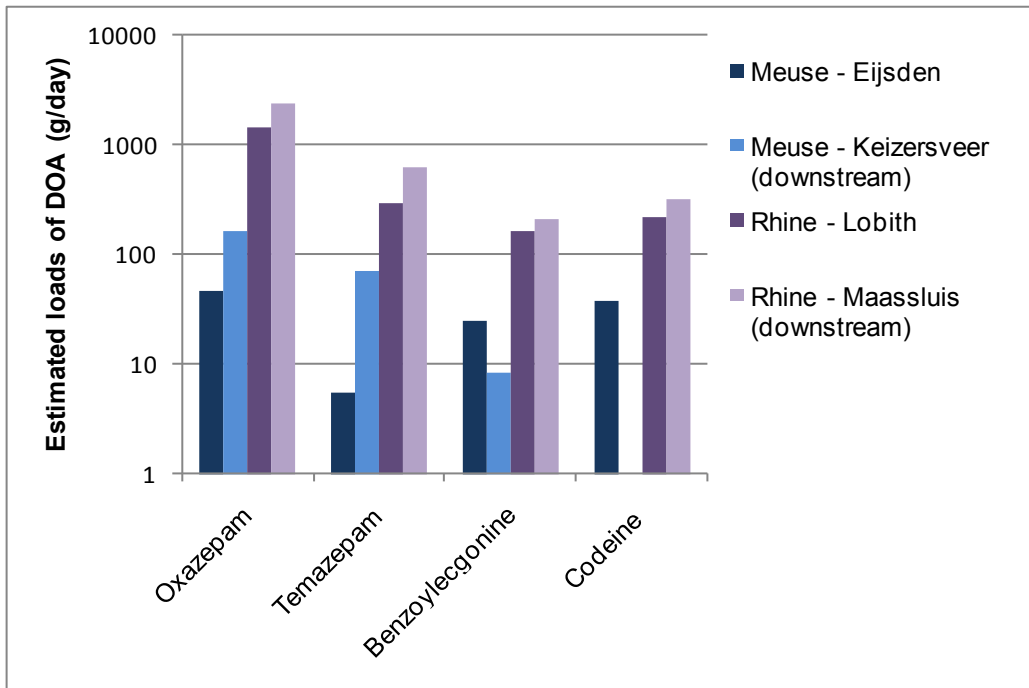
1   **Table 1**: Summary of the analytical methods used by the four laboratories
2

	Sample volume (mL)	Pre-treatment	pH adjustment	SPE column	Type of analytical LC column	Final volume extract (µL)	Injection volume (µL)	Amount of sample analysed (mL)	Conc. factors
RIVM	100	none	No	Oasis HLB (6 cc, 200 mg)	C_{18}	400	25	6.25	250
KWR	900	filtration	pH 7	Oasis HLB (6 cc, 150 mg)	C_{18}	500	20	36	1800
UJI	50	centri-fugation	pH 2	Oasis MCX (6 cc, 150 mg)	C_{18}	1000	20	1	50
UA	50	filtration	pH 2	Oasis MCX (3 cc, 60 mg)	HILIC	200	5	1.25	250

3
4

Table 2: Occurrence of DOA and metabolites in Dutch waters (levels quantified (average ± standard deviation (SD), range and median))

	Influent sewage water				Effluent sewage water				Surface water				Raw drinking- / process water			
		Concentration (ng/L)				Concentration (ng/L)				Concentration (ng/L)				Concentration (ng/L)		
	FD[a]	Average ± SD	Range	Median	FD[a]	Average ± SD	Range	Median	FD[a]	Average ± SD	Range	Median	FD[a]	Average ± SD	Range	Median
Amphetamine	8/8	334 ± 179	107 - 581	310	1/8	15										
Methamphetamine	2/8	151 ± 180	24 - 278	151	4/8	37 ± 20	13 - 62	33	1/14	1						
MDA					1/8	22										
MDMA	8/8	109 ± 51	42 - 207	102	8/8	126 ± 174	17 - 537	56	4/14	2 ± 1	1 - 2	2				
Diazepam					5/8	4 ± 1	2 - 5	3								
Nordazepam					5/8	19 ± 7	13 - 31	18								
Oxazepam	8/8	1167 ± 445	602 - 2020	1105	8/8	1122 ± 375	713 - 1746	959	12/14	29 ± 22	6 - 68	25	7/17	8 ± 5	3 - 13	8
Temazepam	8/8	427 ± 179	255 - 813	411	8/8	568 ± 198	389 - 1016	554	12/14	12 ± 12	3 - 32	6	7/17	4 ± 4	1 - 10	3
THC-COOH	7/8	424 ± 137	289 - 678	378												
Cocaine	8/8	438 ± 245	135 - 904	363	6/8	4 ± 3	1 - 11	3	2/14	2 ± 1	1 - 3	2				
Benzoylecgonine	8/8	1703 ± 870	570 - 2907	1463	8/8	26 ± 25	7 - 84	20	10/14	5 ± 4	1 - 16	3	5/17	2 ± 1	1 - 3	1
Cocaethylene	7/8	27 ± 19	8 - 62	19												
Norbenzoylecgonine	6/8	36 ± 16	18 - 60	38	4/8	4 ± 1	3 - 5	4								
Norcocaine	6/8	20 ± 10	10 - 39	17	1/8	4										
Ecgonine methylester	4/4[b]	207 ± 97	84 - 312	216	3/4[b]	41 ± 2	3 - 6	3								
6-MAM	1/8	3			2/8	5 ± 2	3 - 6	5								
Morphine	8/8	665 ± 418	300 - 1464	517	7/8	31 ± 22	7 - 68	20	1/14	7						
Codeine	8/8	580 ± 230	300 - 975	526	8/8	192 ± 88	110 - 378	168	7/14	7 ± 8	1 - 23	4				
Methadone	4/8	37 ± 20	16 - 64	34	8/8	29 ± 19	6 - 56	22	3/14	2 ± 1	1 - 2	2				
EDDP	4/4[b]	84 ± 41	36 - 135	82	4/4[b]	73 ± 43	25 - 128	67								
Fentanyl					1/8	8										
Ketamine					6/8	16 ± 12	2 - 28	10								
Methcathinone					1/8	4										
Ritalin					6/8	5 ± 3	2 - 9	6								

[a]: Frequency of determination

[b]: Analyzed by UA (STPs: Utrecht, Apeldoorn, Amsterdam, Eindhoven)

Table 3: Environmental Risk Characterization Ratios for eight[a] drugs of abuse

Substance	PNEC (µg/L)	Max. conc. (µg/L) in surface water (MEC)	Environmental Risk Characterization ratio (MEC/PNEC)	
Methamphetamine	2.30[b]	0.001	0.0004	4
MDMA	2.70[b]	0.002	0.0007	5
Oxazepam	4.30	0.068		6
Temazepam	4.30[c]	0.032	Σ 0.0234[d]	
		Σ 0.1[d]		7
Cocaine	4.90[b]	0.003	0.0006	
Benzoylecgonine	4.90[c]	0.016	0.0033	8
Morphine	32.0[b]	0.007	0.0002	9
Codeine	0.06[b]	0.023	0.3800	10

[a] For methadone, which was also detected in surface water (Table 2), no PNEC could be found.

[b] PNEC$_{(ECOSAR)}$, ECOlogical Structure Activity Relationships (ECOSAR) models are used to estimate PNEC.

[c] default PNEC, set at the same level as a related compound with similar metabolic pathway.

[d] sum of oxazepam and temazepam