## Author's response to reviews

**Title:** Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse

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**Author's response to reviews:** see over

Dr. David Ozonoff
Editor-in-Chief
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Dear Dr. Ozonoff,

We have to thank you and your reviewers for the enthusiastic reception you have given our article. Dr. Daughton's comments were particularly constructive and we greatly appreciated the effort he has gone to.
We certainly are very sorry that our paper made Dr. Daughton feel we were trying to take the merit of his proposal published in a symposium series book in 2001. This was not at all our intention, and I think you will see that we did indeed cite his paper and also indicated Dr. Daughton as a competent reviewer. We have now stated more clearly in the revised text that the possibility of using the approach implemented by us had been proposed by Dr. Daughton earlier, and have therefore changed all the sentences where the approach was referred to as "ours".

To avoid any risk of misunderstanding, and make sure both the editors and reviewers understand our point of view, we would, however, like briefly to tell the whole story of how this work started.
Some time ago, while analyzing the levels of pharmaceuticals in the River Po, we found at a certain sampling site strikingly high levels of salbutamol (a bronchodilator). In contrast with what we had observed for most other drugs, these levels could not be explained by local prescription figures (see ref 10). Since salbutamol is sometimes used illegally as an anabolizing agent in farm animals, and the area was rich with farms sending sewage into the river, we started to wonder whether other illegally used drugs could be tracked in the river and urban sewage, and whether their levels could be used to estimate local drug abuse. We chose cocaine as the first drug to test. A literature search immediately found the paper by Dr. Daughton, describing that same idea which, however, had not been implemented.

To get back to the more technical comments, Dr. Daughton's useful points (*reported in Italics*) have been dealt with as follows:

*"The citation for reference #5 has an error (wrong series number) and is also incomplete (missing the title)."*
This citation (now Ref 12) has been corrected.

*"With regard to the technical content of the manuscript, my major reservation derives from the seeming insufficient consideration of the rather complex pharmacokinetics and pharmaodynamics of cocaine."*
We have addressed this issue in the Discussion. See also next paragraph.

*"The key assumption required by the manuscript in back-calculating cocaine (COC, methyl benzoylecgonine) usage rates from environmental residues is the molar mass ratio of the specific COC metabolite benzoylecgonine (BE) to COC itself (namely, BE/COC); incidentally, the prevalent abbreviation used in the literature for cocaine is COC, which should be used in the manuscript. The problem is several fold. The actual ratio for BE/COC used in the manuscript was not statistically derived from the published literature. The manuscript instead used a single ratio from a paper published in 1982. Numerous, more recent references exist regarding the*

*pharmacokinetics of cocaine. A truer (and perhaps statistically valid) value for BE/COC ratio could be synthesized from these."*
We added another reference (see new reference 14: Ambre J. *The urinary excretion of cocaine and metabolites in humans: a kinetic analysis of published data.* J Anal Toxicol. 1985 9:241-5) where cocaine urinary excretion data from the literature were subjected to kinetic analysis, and values were assigned to the various products of cocaine excretion that confirmed our assumptions (unchanged cocaine, 3% and BE, 46%). Other studies may have found somewhat different results, but, having to choose an average value for back-calculations in this first study, we preferred to use these data averaged with a kinetic approach rather than a statistical one. We are aware that this may lead to some inaccuracy in consumption estimates, but this cannot be entirely avoided, as we discuss in the text.

*"Furthermore, COC yields a host of metabolites (and possibly unknown environmental transformation products). The types and relative ratios of metabolites is dramatically affected by the route of exposure: intravenous, intranasal, and pulmonary. For example, one published paper reports that benzoylecgonine can represent 39%, 30%, and 16% of the administered dose for each of these routes, respectively. The important point is that the ratio of BE to COC is not constant, and therefore BE cannot necessarily be used to back-calculate to a give an estimate as accurate as portrayed in the manuscript."*
We have now discussed the intrinsic limitation of our data in this respect (see Discussion). We therefore stated that the assumption made here may lead to some inaccuracy in consumption estimates.

*"To make matters even more complicated, the molar ratio of BE/cocaine (resulting from urine) could change radically after sewage treatment and after environmental processes act upon them, as it is unknown whether cocaine would be preferentially biodegraded versus its metabolites. If these problems cannot be addressed, then the manuscript needs to discuss these limitations in more depth. Instead of trying to decipher exactly what percentage of the original overall COC usage the measured BE accounted for, another approach would have been to perform a more comprehensive analysis for combined COC, BE, and other metabolites, including ecgonine methyl ester, norcocaine, benzoylnorecgonine, m-hydroxycocaine, p-hydroxycocaine, m-hydroxybenzoylecgonine, and p-hydroxybenzoylecgonine. In the published study just mentioned, these additional metabolites combined accounted for 18%, 15% and 8% of the administered dose for intravenous, intranasal, and pulmonary routes, respectively. By measuring as many metabolites (and residual, parent COC) as possible, a more accurate (but minimum) estimate of COC could be calculated without making assumptions requiring ratios."*
We agree that more comprehensive analysis of cocaine metabolites would lead to more accurate estimates of consumption, but this was beyond the scope of this first study. In any case, even in a more refined version of this approach for drug abuse trends monitoring at given WWTPs, measurement of many more metabolites would add greatly to the costs and time of analysis without significant advantages in the quality of results. Clearly, in cases where stringent accuracy of estimates were of utmost importance, a thorough analysis of all known metabolites of the drug under study would be desirable.

*"Two statements on page 4 seem to be contradictory and therefore need to be clarified:*
*(1) "Our approach implies that consumption figures are very unlikely over-estimated."*

*(2) "...we must conclude that our estimates of cocaine consumption could not over-estimate true values."*
*Statement 1 in particular seems be worded incorrectly. It seems that not only the usage figures provided by traditional paper surveys UNDER-estimate usage, but that the values calculated from the actual monitoring data probably also UNDER-estimate usage. This needs to be clarified."*
The text has been changed and expanded to make this point clearer (see Background, and Discussion)

*"Another major point of confusion in the manuscript is where exactly the WWTP sampling was performed. On page 5, the very confusing noun "affluent" is used. Does "affluent" refer to where the treated sewage EFFLUENT connects with the receiving stream? This would be the only proper use for the word "affluent," which just isn't used when describing sewage treatment. Instead, the words "influent" or "effluent" should be used, depending on which stream is being described. If the effluent (treated sewage) were sampled, then the values for BE and COC are further biased since an unknown (but perhaps significant amount) of BE or COC could have been removed or transformed. Alternatively, BE could have been produced by COC. The BE/COC ratio could have been changed by the waste treatment process itself. COC, because of its higher octanol-water coefficient, could have partitioned to the sewage sludge. By sampling the influent, the values obtained for COC, BE, or any other metabolite would be the most representative of the values existing in human urine."*
We are aware that concentrations of urinary excretion products of cocaine are better represented by waste water taken before it enters WWTP, and therefore used the influent and not the effluent for this study. "Affluent" is in fact another (rarer) term for "influent". The latter term has now been used throughout the text.

*"By taking samples from the River Po, which receives discharged treated sewage, the total mass fluxes of cocaine and its metabolites will be lower than those entering the sewage treatment plant because portions (of unknown magnitude) of each will be removed by the sewage treatment processes."*
We agree that river water concentrations are theoretically less useful for predictions, however there is the possibility that – as described for some pharmaceuticals – BE is not removed by treatment plants, is distributed preferentially in water, and is "stable" in this aqueous environment. This hypothesis has to be verified, but it is clearly possible since, as specified in the text, we found a "fair correspondence of SW and WW findings" regarding cocaine consumption estimates back-calculated from BE levels. On the contrary, since the ratio cocaine/BE was lower in river than in influents of WWTPs, it might be that cocaine is removed by WWTPs, and/or differently partitioned and/or less stable in water than BE.

*"Regardless of the usefulness of river water concentrations for predicting overall usage rates, it is nonetheless very significant that cocaine can be repeatedly detected in a major river. This point should be emphasized in the manuscript."*
We agree that the repeated detection of cocaine, and BE, in river water is a significant point, and we now emphasized it in the text.

*"The relative distribution coefficients (between the aqueous phase and the sewage sludge, suspended solids, or sediments) for cocaine versus its metabolites is unknown. What portion of each would be expected to partitioned to the sewage*

*solids and remain with the solids? This could account for the lower than expected ratio of cocaine to BE (page 7).”*
See previous point.

*“Questions concerning the environmental fate of cocaine are germane to the statement beginning on page 7: "Moreover, having chosen in our study to monitor an abundant metabolite in addition to the parent drug, any increase in cocaine levels due to illicit disposal rather than human use would be promptly disclosed by an increased cocaine/metabolite ratio." This statement might be true if the monitoring were done on the untreated influent to the sewage treatment plants. But cocaine disposal could itself lead to formation in the sewage treatment process or in the environment of the some of the same cocaine metabolites (e.g., BE) as created by human metabolism. Analysis of treated sewage effluent would not be able to distinguish these.”*
As specified above, we sampled the influent.

*“Using the examples provided above, the manuscript should devote significant discussion to some of the pitfalls involved in the assumptions that were made.”*
This has been now addressed in the Discussion.

*“The manuscript also needs to provide more specifics regarding a wide range of additional questions that currently cannot be answered by reading the manuscript. For example, several parameters were essential to the calculation of cocaine usage. How was River Po's flow rate determined at the actual sampling time? Please show the dimensional analyses (unit conversions) that were used in converting the concentrations of the analytes in the samples to the total usage.”*
We obtained mean flow rates for the sampling period by the “Ufficio mareografico ed idrografico del Po”, which is a public office in charge of such measurements.
The unit of conversion used are specified in the text (see Methods, under “Sample collection” and “Calculations and assumptions”).

*“The manuscript is deficient in discussion of quality assurance. Some of the items that need to be addressed include: (1) What types of blanks were analyzed (travel blanks, field blanks, instrument blanks, etc.). (2) Were spiked samples used to determine analyte stability? Was a sample holding time study performed? How long were samples stored? Analyte loses during storage would yield negatively biased results. (3) How was analyte identification performed? How did MS m/z values and relative m/z abundances of the unknowns compare with the authentic standards? (4) No purity was stated for the authentic standards. Were any steps taken to assess the purity? (5) No operating conditions were provided for the MS/MS experiments. (6) What criteria were used to calculate the limits of detection?”*
The section “Methods” has been extended, giving many more experimental details and addressing the above questions.

*“Page 6: The term "lines" as in "(four 25-mg lines of cocaine)" is jargon and may not be understood by some readers”.*
We used this phrase to help the reader “visualize” a 100-mg cocaine dose. The word “lines” in now within quotation marks.

*“Page 8: Is the following statement a published statistic or one that is being inferred from the work here? "The 1500 kilograms of cocaine supposedly consumed per year in the River Po basin""*
“1500 kilograms of cocaine” is the value estimated by us, as now more clearly specified in the text.

*“Table 1: The title ("Levels and loads of cocaine and its metabolite in the River Po and WWTPs") gives the impression that the metabolite listed in the table (BE) is the ONLY COC metabolite. Since COC has numerous metabolites, the specific one being targeted needs to be specified. A suggested alternative title is: "Levels and loads of cocaine and its metabolite (benzoylecgonine, BE) in the River Po and WWTPs". Also, do the data presented in Table 1 justify the number of significant figures displayed? Since these values are merely estimates based on a single assumed ratio of BE/COC (whose accuracy is questionable), the number of significant figures is certainly fewer than 4 and perhaps even fewer than 2. Table 2: This table needs to make clear that the estimates are based on the concentrations of BE and COC actually measured in the waters. And as in Table 1, given the uncertainties in the calculations, how many significant figures are justified for the data?”*
We changed the title of Tables 1 and 2, as suggested. We have now used a minimal number of significant figures.

As to Dr. Voulvoulis’ comments, we have addressed the various points (**reported in bold below**) as follows:

**“Investigators may require a little more detail of the method in order to repeat the study.”**
The experimental work has been now thoroughly described.

**“Discussion is brief.”**
The discussion section has been now extended.

**“There could be more on the limitations of the study. Possibly some mention of the environmental half-life of cocaine and BE.**”
Unfortunately, there are no data on this subject, to our knowledge. This will have to be addresses in future studies.

**“Could be a comparison of the predicted and measured environmental concentrations and prescription numbers of legal drugs - authors' own work is cited but perhaps they could also look elsewhere for further evidence of the efficacy of the method.”**
Another reference (new ref. 12) on this type of comparison has been added.

Overall, we have added eight new references to the text. Please let us know if we should send by email a copy of the manuscript with changes/additions marked in red, to facilitate locating them in the revised text.

We hope to have satisfactorily addressed the reviewer’s concerns, and that our manuscript is now suitable for publication in EH.

We thank you and your reviewers for considering our work, and for giving us the opportunity to improve the quality of the manuscript.

Looking forward to hearing from you,

Sincerely,

Ettore Zuccato