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Article

Estimation of Equivalent Cutoff Thresholds in Blood and Oral Fluid for Drug Prevalence Studies

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Oral fluid is an easily available specimen for studying drug use in a cohort or population. The prevalence of drugs in samples of oral fluid is the same as the prevalence in blood if using equivalent cutoff concentrations. The cutoffs in oral fluid may be higher or lower than that in blood in accordance with the median oral fluid-to-blood (OF/B) concentration ratio, but it is also influenced by the skewness of the distribution of OF/B ratios. The aim of this study was to determine formulae for the estimation of equivalent cutoff concentrations in oral fluid and blood for 12 commonly used illegal and medicinal psychoactive drugs when oral fluid was collected with Statsure Saliva·Sampler™. Paired samples from 4,080 persons were collected and analyzed with chromatographic methods and mass spectroscopic detection. Regression formulae for the concentrations corresponding to selected percentiles in oral fluid versus the same concentration percentiles in blood were determined. The accuracy when multiplying the cutoff thresholds in blood with the average and median OF/B ratios to estimate equivalent cutoffs in oral fluid was also investigated. Prevalence regression gave the most accurate results. The regression formulae can be used to estimate equivalent cutoff concentrations in oral fluid and blood.

Introduction

Oral fluid (mixed saliva) is increasingly used for drug testing in epidemiological studies of drug use. Oral fluid can easily be collected using commercially available sampling devices, and the sampling time is just a few minutes (1). The sampling is less intrusive than sampling of blood, hair and urine; therefore, the participation rate in epidemiological studies has been higher when collecting oral fluid than when collecting other biological specimens. Recent studies have obtained refusal rates as low as 3–6% when collecting oral fluid (2–4), whereas previously reported refusal rates were 24–60% when collecting blood samples (5–7), 24% for urine samples (8) and 14–44% for hair samples (8, 9); in addition, many had insufficient hair for sampling. Monetary incentives were offered in most of the studies collecting blood, urine or hair.

A drug finding in oral fluid is a strong indication of recent drug intake, and a blood sample taken at the same time will usually also be positive for the actual drug if using appropriate analytical cutoff thresholds for both matrices (10). The collection of oral fluid is therefore an excellent tool for studying recent drug use in a cohort or population; however, for most drugs the concentration in oral fluid cannot be used to accurately estimate the drug concentration in blood for an individual because of large interindividual variations in concentration ratios between oral fluid and blood (11–13). The calculated relative standard deviations for oral fluid-to-blood (OF/B) ratios are often 50–100%.

For a cohort of drug users, the statistical distribution of drug concentrations in blood and in oral fluid will reflect the use of drugs by the individuals in the cohort. It is therefore expected that the drug concentrations in samples of oral fluid from the cohort can be used to estimate data on blood drug concentrations of the cohort.

If analyzing the samples of blood and oral fluid from all individuals in the cohort, the number of positive drug findings in blood and oral fluid will not always be equal because drug concentrations are different in blood and oral fluid and the analytical methods might not be sufficiently sensitive to detect all positive cases in both types of specimen. However, when using 'equivalent' cutoff concentrations, this difference will be minimized or eliminated. Equivalent cutoff concentrations mean that the prevalence of drug concentrations above cutoff C_B in blood will be equal to that of drug concentrations above cutoff C_{OF} in oral fluid in the studied cohort. Equivalent cutoff thresholds also imply that both specimens will, on average, be positive for a drug for the same length of time following the intake of a single drug dose. The equivalent cutoff concentration in oral fluid may be higher or lower than that in blood in accordance with the median OF/B concentration ratio. Owing to interindividual variations, equivalent cutoff concentrations should not be used for assessing drug concentrations in oral fluid from single individuals, only in cohorts of drug users.

We have previously found that multiplying the blood concentration C_B with the OF/B regression coefficient (slope) or the average OF/B ratio gives a rough estimate of the equivalent concentration C_{OF} in oral fluid for amphetamine and tetrahydrocannabinol (THC) (14). The actual differences between equivalent drug concentration cutoffs C_B and C_{OF} are related not only to the mean OF/B ratio, but also to the statistical distribution of OF/B ratios among drug users. Therefore, more accurate estimates for equivalent cutoff concentrations of blood and oral fluid may be obtained by using prevalence regression of aggregated population data (15) or a statistical simulation method (14), which is a more challenging procedure.

In some previous cross-sectional studies of drug use, participants were asked to provide either a sample of blood or a sample of oral fluid (6, 16). Analytical results from blood and oral fluid samples were pooled and used to calculate the overall prevalence of drug use. In some previous case–control studies, samples of blood were collected from cases and oral fluid from

controls (17–19). In those types of studies, equivalent cutoff thresholds must be used in order to make sound estimations of total drug prevalence or sound estimates of odds ratios in case–control studies.

The aim of this study was to determine formulae for the estimation of equivalent drug cutoff concentrations in whole blood and oral fluid collected with Statsure Saliva·Sampler™.

Materials and methods

Samples

A total of 4,080 paired samples of whole blood and oral fluid were collected from drivers in Belgium ($n = 2750$), Finland ($n = 339$), Italy ($n = 891$) and Norway ($n = 100$) during 2007–2010. This cohort consisted partly of random drivers included in a roadside survey of alcohol, drugs and driving; partly of injured drivers admitted to the hospital after involvement in a traffic accident and partly of drivers arrested by the police suspected for drug driving. Samples of oral fluid were collected using Statsure Saliva·Sampler™ (Saliva Diagnostic Systems, Framingham, MA, USA), whereas whole blood was sampled using tubes containing potassium oxalate and sodium fluoride. All paired samples of blood and oral fluid were collected within 30 min. Samples of oral fluid were stored at 2–8°C for a maximum of 48 h and thereafter frozen at about −20°C until analyzed. Most drugs were found to be stable in collected oral fluid at 2–8°C for a week (20). For blood samples, handling and storage was done in accordance with local routines. In general, samples might have been kept at ambient temperature between sampling and delivery to the analytical laboratory up to 3 days. When received by the laboratory, samples were either frozen or stored at 2–8°C for up to 2 weeks before being frozen at about −20°C. The added preservatives ensured that degradation was minimized.

Analytical methods

Samples of blood and oral fluid were analyzed with high-performance or ultra performance liquid chromatography or gas chromatography with single or tandem mass spectrometric detection using validated analytical methods in Belgium (21), Norway (22), Italy (23, 24) and Finland (25, 26) as previously described. Only quantitative data above the limits of quantification (LOQs) for the participating countries (12) were included. Analytical findings for cocaine and oxazepam from the Norwegian laboratory were not included for prevalence regression calculations, because the cutoff concentrations used for blood samples were significantly higher than those used by the other participating laboratories (12).

Estimation of equivalent cutoff concentrations in blood and oral fluid

For each drug, the cohort of drug-positive cases was defined as individuals who had positive findings of the drug in question in oral fluid, blood or both. We studied the accuracy of three methods for determining equivalent cutoff concentrations in oral fluid and blood: multiplying the cutoff concentration in blood with the average or median OF/B ratio (12) and the use of prevalence regression (15) to determine formulae defining the relations between the prevalence in samples of blood and oral fluid.

For prevalence regression, concentrations in oral fluid corresponding to the 40th, 45th, 50th, 55th, 60th, 65th, 70th, 75th, 80th, 85th, 90th and 95th percentiles were plotted against the drug concentrations in blood corresponding to the same percentiles. Outliers were not excluded when calculating the percentiles. If only blood or oral fluid was positive (i.e., the concentration was above the analytical cutoff), the other specimen was given a concentration of zero for the prevalence regression calculations.

If any of the lower percentile concentrations were lower than the analytical cutoff concentration for either oral fluid or blood used by any of the participating laboratories, those percentiles were deleted from the analysis.

Regression curve equations were determined using the 'trendline' function in Microsoft Excel. Linear, exponential, quadratic and power functions were calculated, and the regression formula with highest correlation coefficient was selected for each drug. The obtained formula thus described the relationship between equivalent concentration percentiles in oral fluid and blood. Thereby, the prevalence (or percentile) of drug concentrations above a given cutoff threshold in blood would be equal to that of drug concentrations in oral fluid above a concentration calculated by using the regression formulae.

For each drug, the accuracy for the determination of prevalence above low, medium and high concentration thresholds were studied for the three estimation methods. As low thresholds, the cutoff concentrations for blood used in the European project 'Driving under the Influence of Drugs, Alcohol and Medicines' (DRUID) (16) and recommendations from an international expert meeting (27) were selected. The high concentration thresholds were selected so that 10–15% of the found drug concentrations in the analyzed samples were above this threshold, and the medium concentration thresholds corresponded to 50% of the high concentration thresholds.

Differences between estimated and actual drug prevalence above the low, medium and high concentration thresholds for the three estimation methods were analyzed using a Student's *t*-test.

Approximate 95% confidence intervals (95% CIs) for cutoff concentrations in oral fluid that were equivalent to the low cutoff threshold for blood were estimated by first calculating the 95% CI quantiles p using the Wald method (28): $p = p' \pm 1.96 \cdot [p'(1 - p')/n]^{\frac{1}{2}}$, where p' is the prevalence (percentile) of blood concentrations above the low cutoff threshold, and then calculating the drug concentrations in oral fluid corresponding to the quantiles p using the formula that gave the best accuracy.

Results

In total, alprazolam was detected in samples of blood and/or oral fluid from 106 subjects, amphetamine 86, clonazepam 57, cocaine 112, codeine 92, diazepam 94, methamphetamine 55, morphine 76, nordiazepam 130, oxazepam 55, THC 182 and tramadol in 51 subjects.

The accuracy obtained when using the three methods for determining equivalent cutoff concentrations in oral fluid and blood are presented in Table I. Prevalence regression gave

Table I
Comparison of the accuracy for estimated equivalent cutoff concentrations in oral fluid

Substance (number of cases)	Concentrations in blood (ng/mL)	Actual prevalence in blood (%)	Estimated prevalence in blood (and accuracy) using oral fluid (%)		
			PR	AV	ME
Alprazolam ($n = 106$)	≥10	39.6	38.7 (−0.9)	30.2 (−9.4)	34.0 (−5.6)
	≥25	22.6	26.4 (+3.8)	20.8 (−1.8)	21.7 (−0.9)
	≥50	13.2	13.2 (+0.0)	13.2 (+0.0)	15.1 (+1.9)
			Δ = 1.6	Δ = 3.7	Δ = 2.8
Amphetamine ($n = 86$)	≥25	62.8	55.8 (−7.0)	51.2 (−11.6)	52.3 (−10.5)
	≥200	25.6	26.7 (+1.1)	25.6 (+0.0)	27.9 (+2.3)
	≥400	11.6	11.6 (+0.0)	14.0 (+2.4)	16.3 (+4.7)
			Δ = 2.7	Δ = 4.7	Δ = 5.8
Clonazepam ($n = 57$)	≥10	56.1	56.1 (+0.0)	43.9 (−12.2)	50.9 (−5.2)
	≥25	28.1	31.6 (+3.5)	26.3 (−1.8)	33.3 (+5.2)
	≥50	10.5	7.0 (−3.5)	12.3 (+1.8)	15.8 (+5.3)
			Δ = 2.3	Δ = 5.3	Δ = 5.2
Cocaine ($n = 112$)	≥10	23.2	23.2 (+0.0)	23.2 (+0.0)	24.1 (+0.9)
	≥40	16.1	17.9 (+1.8)	10.7 (−5.4)	10.7 (−5.4)
	≥80	13.4	10.7 (−2.7)	8.0 (−5.4)	8.0 (−5.4)
			Δ = 1.5	Δ = 3.6	Δ = 3.9
Codeine ($n = 92$)	≥10	31.5	33.7 (+2.2)	31.5 (+0.0)	46.7 (+15.2)
	≥20	23.9	21.7 (−2.2)	19.6 (−4.3)	28.3 (+4.4)
	≥40	14.1	12.0 (−2.1)	9.8 (−4.3)	15.2 (+1.1)
			Δ = 2.2	Δ = 2.9	Δ = 6.9
Diazepam ($n = 94$)	≥50	53.2	54.3 (+1.1)	47.9 (−5.3)	53.2 (+0.0)
	≥125	39.4	39.4 (+0.0)	27.7 (−11.7)	40.4 (+1.0)
	≥250	13.8	12.8 (−1.0)	14.9 (+1.1)	22.3 (+8.5)
			Δ = 0.7	Δ = 6.0	Δ = 3.2
Methamphetamine ($n = 55$)	≥25	70.9	72.7 (+1.8)	72.7 (+1.8)	74.5 (+3.6)
	≥300	36.4	32.7 (−3.7)	27.3 (−9.1)	30.9 (−5.5)
	≥600	14.5	12.7 (−1.8)	12.7 (−1.8)	20.0 (+5.5)
			Δ = 2.4	Δ = 4.2	Δ = 4.9
Morphine ($n = 76$)	≥10	42.1	36.8 (−5.3)	36.8 (−5.3)	44.7 (+2.6)
	≥20	18.4	25.0 (+6.6)	25.0 (+6.6)	31.6 (+13.2)
	≥40	13.2	10.5 (−2.7)	15.8 (+2.6)	21.1 (+7.9)
			Δ = 4.9	Δ = 4.8	Δ = 7.9
Nordiazepam ($n = 130$)	≥50	50.8	46.2 (−4.6)	44.6 (−6.2)	46.2 (−4.6)
	≥150	26.2	27.7 (+1.5)	26.9 (+0.9)	31.5 (+5.3)
	≥300	13.1	10.8 (−2.3)	14.6 (+1.5)	16.9 (+3.8)
			Δ = 2.8	Δ = 2.9	Δ = 4.6
Oxazepam ($n = 55$)	≥50	25.5	23.6 (−1.9)	30.9 (+5.4)	34.5 (+9.0)
	≥125	16.4	18.2 (+1.8)	20.0 (+3.6)	27.3 (+10.9)
	≥250	10.9	9.1 (−1.8)	18.2 (+7.3)	9.1 (+9.1)
			Δ = 1.8	Δ = 5.4	Δ = 9.7
THC ($n = 182$)	≥1.0	28.6	28.6 (−0.0)	33.5 (+4.9)	44.5 (+15.9)
	≥1.5	24.7	23.6 (−1.1)	27.5 (+2.8)	38.5 (+13.8)
	≥3.0	13.7	12.6 (−1.1)	21.4 (+7.7)	30.2 (+16.5)
			Δ = 0.7	Δ = 5.1	Δ = 15.4
Tramadol ($n = 51$)	≥25	35.3	33.3 (−2.0)	31.4 (−3.9)	33.3 (+2.0)
	≥100	23.5	23.5 (+0.0)	29.4 (−3.9)	19.6 (−3.9)
	≥200	11.8	13.7 (+1.9)	5.9 (−5.9)	9.8 (−2.0)
			Δ = 1.3	Δ = 4.6	Δ = 2.6
Average deviation	–	–	Δ = 2.1	Δ = 4.4	Δ = 6.1

Deviations from actual prevalence are within parentheses and average of the absolute values of deviations is represented as Δ.
PR, prevalence regression; AV, multiplication of the concentrations in blood with the average OF/B concentration ratio; ME, multiplication with the median OF/B ratio (12).

the best accuracy for all drugs studied except for morphine; statistically significant differences were found when studying the absolute values of the deviations from actual prevalence for prevalence regression compared with using the average or median multiplications ($P < 0.001$). The results thus indicate that prevalence regression is the most robust method for the determination of equivalent cutoff concentrations in oral fluid and blood. Examples of prevalence regression curves are presented in Figure 1.

The estimated cutoff concentrations in oral fluid equivalent to those used for blood in the DRUID project are presented in Table II together with 95% CI and the best fitting formulae for estimating equivalent cutoff concentrations.

The accuracy of the best fitting formulae was also studied by comparing the actual prevalence of drug concentrations above the low cutoff concentrations in blood from the studied cohort of 4,080 subjects with estimated prevalence including 95% CI based on the analysis of oral fluid. Results are presented in Table III. For clonazepam and diazepam, the CIs could not be calculated because the lower 95% CI concentration was below the quantification limits of the analytical methods. The accuracy was good and the 95% CI acceptable.

Discussion

Previous studies using the same paired samples of oral fluid and blood as those used in this study found that the concentrations in oral fluid cannot be used to accurately estimate the drug concentration in blood for individuals because of large variations in concentration ratios between oral fluid and blood (12, 13). However, other studies have found that when

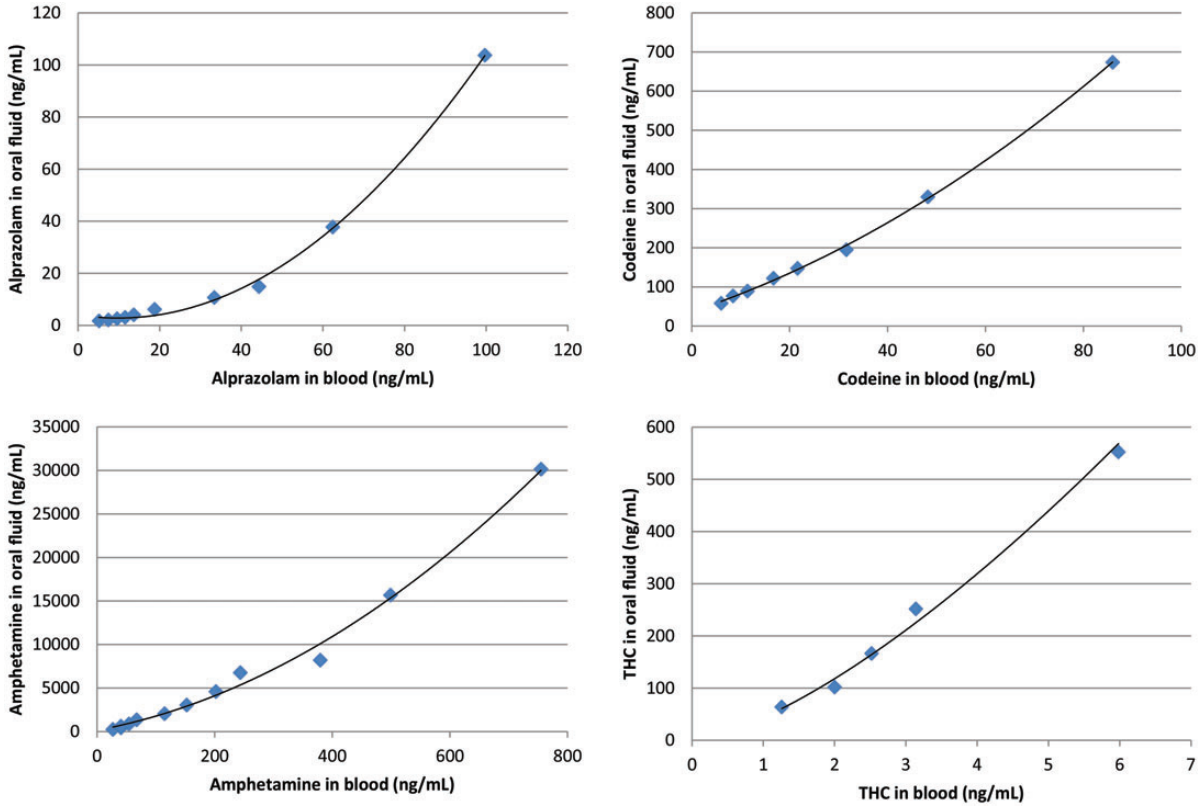

Figure 1. Prevalence regression curves for alprazolam, amphetamine, codeine and THC. Every 5th percentile from the 40th to the 95th is plotted, except for percentiles corresponding to concentrations below the analytical cutoff concentrations for the analytical methods.

Table II
Estimated equivalent cutoff concentrations in blood and oral fluid, formulae, and coefficients of determination

Substance	Cutoff in whole blood (ng/mL)	Cutoff in oral fluid[a] [95% CI] (ng/mL)	Prevalence regression formulae	R^2
Alprazolam	10	2.8 [1.8–4.2]	$C_{OF} = 0.013 \times C_B^2 - 0.25 \times C_B + 4$	0.998
Amphetamine	20	290 [84–680]	$C_{OF} = 0.034 \times C_B^2 + 13.7 \times C_B$	0.993
Clonazepam	10	1.2 [0.2–2.0]	$C_{OF} = 0.56 \times 2.718^{0.079 \times C_B}$	0.962
Cocaine	10	190 [26–350]	$C_{OF} = 161 \times 2.718^{0.019 \times C_B}$	0.932
Codeine	10	83 [50–130]	$C_{OF} = 0.038 \times C_B^2 + 4.2 \times C_B + 37$	0.999
Diazepam	50	1.1 [0.3–3.6]	$C_{OF} = 0.00024 \times C_B^2 + 0.0089 \times C_B$	0.930
Methamphetamine	20	630 [120–1800]	$C_{OF} = 0.033 \times C_B^2 + 8.0 \times C_B + 891$	0.993
Morphine	10	100 [37–180]	$C_{OF} = 9.83 \times C_B$	0.902
Nordiazepam	50	2.2 [1.2–4.5]	$C_{OF} = 0.000085 \times C_B^2 + 0.040 \times C_B$	0.997
Oxazepam	50	12 [4.4–34]	$C_{OF} = 0.235 \times C_B$	0.962
THC	1.0	44 [27–90]	$C_{OF} = 44 \times C_B^{1.4}$	0.991
Tramadol	50	490 [85–1500]	$C_{OF} = 9.74 \times C_B$	0.966

C_{OF}, drug concentration in oral fluid; C_B, drug concentration in blood.
[a]Rounded to two significant digits.

studying aggregated concentration data for a cohort, acceptable accuracies on drug use in the cohort in total may be obtained (14, 15), in spite of the fact that findings for single individuals in the cohort may be incorrect. We expected that the prevalence regression method would give the most accurate calculations of equivalent cutoff concentrations, because the skewness of the statistical distribution of OF/B ratios gives a non-linear regression curve (15). The average OF/B ratio may, however, be used as approximations for some drugs or for limited concentration intervals for other drugs, as previously found for amphetamine and THC (14), albeit the estimated prevalence of high drug concentrations in blood may be inaccurate when using the average (14). It is also expected that the accuracy of the estimation is related to the number of subjects in the studied cohort due to large interindividual variations; the larger the number of individuals, the better the accuracy.

Previously, multiplication of the cutoff concentration in blood with the slope of the linear regression between oral fluid and blood has also been suggested as a method for estimating the equivalent cutoff in oral fluid. However, single extreme values have a large effect on the calculated coefficient, and initial calculations using the data presented in this study indicated that the accuracy therefore was poor (results not shown).

Table III
Actual and estimated prevalence (%) of blood drug concentrations above cutoffs in blood samples from the studied cohort of 4,080 individuals using the best fitting formulae

Substance	Actual prevalence above cutoffs in blood	Estimated prevalence above cutoffs in blood based on concentrations in oral fluid [95% CI]
Alprazolam	1.0	1.0 [0.8–1.3]
Amphetamine	1.3	1.3 [1.0–1.5]
Cocaine	0.6	0.7 [0.4–0.9]
Codeine	0.7	0.8 [0.5–1.0]
Methamphetamine	1.0	1.0 [0.8–1.2]
Morphine	0.8	0.7 [0.5–0.9]
Nordiazepam	1.6	1.5 [1.2–1.7]
Oxazepam	0.3	0.3 [0.2–0.5]
THC	1.2	1.3 [1.0–1.6]
Tramadol	0.4	0.4 [0.2–0.5]

For all drugs except morphine, prevalence regression fitted the data better than using the mean or median OF/B ratios for estimating equivalent cutoff concentrations in oral fluid and blood. This finding complies with the results of a previous study of amphetamine and THC (15); however, the regression curves were somewhat different, primarily because a different type of oral fluid collection device was used.

The 95% CI for estimated cutoff concentrations in oral fluid seems to be wide (Table II). However, due to the very wide distribution of drug concentrations in oral fluid, the 95% CI for the estimated prevalence in blood based on the analysis of oral fluid was acceptable as shown in Table III. For example, the 95% CI for the cutoff for amphetamine in blood was 84–680 ng/mL, which corresponded to an amphetamine prevalence of 1.0–1.5% in the studied population (i.e., 1.5% had concentrations above 84 ng/mL and 1.0% above 680 ng/mL in oral fluid).

Results of preliminary estimations (29) were used in the DRUID project to estimate cutoff concentrations in oral fluid that were equivalent to those used for blood. These data were used when calculating the prevalence of drugs in blood and oral fluid samples from drivers in 13 European countries (7, 30). The estimated cutoff concentrations for oral fluid were based on a smaller set of data, and linear regression lines were prioritized whenever possible. In addition, estimated drug concentrations below the analytical cutoff concentrations were included in the original estimations; those were not included when calculating the cutoff concentrations for oral fluid presented in Table II. Therefore, the results presented in this article are somewhat different for some substances, but probably more accurate.

When studying the prevalence of drugs in blood or oral fluid samples collected from a cohort, it is important to choose cutoff concentrations that are relevant to what the intention is to study, such as either any recent drug use (using a very low cutoff concentrations) or drug use that may cause impairment (higher cutoffs). Using alcohol as an analog, if the cutoff is 0.1 g/L for alcohol, the prevalence of positive findings will be much higher than if using a cutoff of 0.5 g/L, which is the legal limit for drunk driving in most European countries.

The cutoff concentrations for blood presented in Table II were primarily based on analytical capabilities and not on pharmacological effects. The prevalence of drug concentrations above those cutoff limits can therefore not be used to compare different drugs regarding the prevalence of impairment or misuse, neither if analyzing blood nor if analyzing oral fluid samples. An attempt was made to define drug concentration cutoffs similar to a blood alcohol concentration of 0.2 g/L when determining the Norwegian legal limits for driving under the influence of drugs. Those drug concentration limits were either defined as one-fifth of the maximum concentration in whole blood observed after the use of 'standard' recreational and inebriating drug doses or determined by assessing the impairment corresponding to an alcohol concentration of 0.2 g/L after single drug doses in naive individuals (31).

The formulae presented in Table II may then be used to estimate cutoff concentrations in oral fluid collected with the Statsure device that are equivalent to chosen cutoff concentrations for blood. A limitation is that the formulae may not apply for oral fluid collected with other types of devices. The formulae are not intended for the assessment of drug concentrations in oral fluid for single individuals due to the large interindividual variation in OF/B ratios observed.

Another limitation of the study was that the formulae that fitted the original data best were regarded as the most accurate ones without validating the formulae using data from an independent cohort. No other large study has collected oral fluid with the Statsure device and simultaneously collected paired samples of blood; therefore such a validation or comparison with other cohorts could not be performed. We assume that such a study would probably find somewhat larger inaccuracies and wider 95% CI for the estimated cutoff concentrations in oral fluid.

The best option in cross-sectional studies and case–control studies of drugs of abuse would be to avoid using more than one type of biological matrix for drug analysis. In studies of drug-related impairment and in case–controls studies of drug-related risk for involvement in traffic crashes, blood samples should be the matrix of choice if it is possible to obtain high participation rate. At present, this seems to be difficult; therefore, collection of blood from 'cases' and oral fluid from 'controls' has been chosen in several studies. It is then important to use equivalent cutoff concentrations for drugs in those two matrices. In the future, the use of microsampling techniques for blood (32) might become a better option than collecting oral fluid from controls if it is possible to obtain a higher participation rate than when using traditional blood sampling.

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Conflict of interest

This document reflects only the authors' view. The European Commission is not liable for any use that may be made of the information contained herein.

References

1. Langel, K., Engblom, C., Pehrsson, A., Gunnar, T., Ariniemi, K., Lillsunde, P. (2008) Drug testing in oral fluid—evaluation of sample collection devices. *Journal of Analytical Toxicology*, **32**, 393–401.
2. Gjerde, H., Christophersen, A.S., Moan, I.S., Yttredal, B., Walsh, J.M., Normann, P.T. *et al.* (2010) Use of alcohol and drugs by Norwegian employees: a pilot study using questionnaires and analysis of oral fluid. *Journal of Occupational Medicine and Toxicology*, **5**, 13.
3. Gjerde, H., Christophersen, A.S., Normann, P.T., Assum, T., Pettersen, B.S., Rognerud, A.J. *et al.* (2011) Country report Norway. In Houwing, S., Hagenzieker, M., Mathijssen, R., Bernhoft, I.M., Hels, T., Janstrup, K. (eds) *Prevalence of Alcohol and Other Psychoactive Substances in Drivers in General Traffic. Part II: Country Reports. DRUID Deliverable D 2.2.3.* SWOV Institute for Road Safety Research, Leidschendam, The Netherlands, pp. 152–163. http://orbit.dtu.dk/fedora/objects/orbit:89805/datastreams/file_6418383/content (accessed Sept 9, 2013).
4. Gjerde, H., Christophersen, A.S., Normann, P.T., Pettersen, B.S., Sabaredzovic, A., Samuelsen, S.O. *et al.* (2011) Analysis of alcohol and drugs in oral fluid from truck drivers in Norway. *Traffic Injury Prevention*, **13**, 43–48.
5. Lacey, J.H., Kelley-Baker, T., Furr-Holden, D., Brainard, K., Moore, C. (2007) *Pilot test of new roadside survey methodology for impaired driving. DOT HS 810 704.* National Highway Traffic Safety Administration, Washington, DC http://www.nhtsa.gov/people/injury/research/pub/HS810704/Pilot-Test-of_NRSM.pdf (accessed Sept 9, 2013).
6. Lacey, J.H., Kelley-Baker, T., Furr-Holden, D., Voas, R.B., Romano, E., Ramirez, A. *et al.* (2009) *2007 National Roadside Survey of Alcohol and Drug Use by Drivers—Drug Results. DOT HS 811 249.* National Highway Safety Administration, Washington, DC http://www.nhtsa.gov/DOT/NHTSA/Traffic%20Injury%20Control/Articles/Associated%20Files/811249.pdf (accessed Sept 9, 2009).
7. Houwing, S., Hagenzieker, M., Mathijssen, R., Bernhoft, I.M., Hels, T., Janstrup, K. *et al.* (2011) *Prevalence of alcohol and other psychoactive substances in drivers in general traffic. Part I: General results. DRUID Deliverable D 2.2.3.* SWOV Institute for Road Safety Research, Leidschendam, The Netherlands. http://orbit.dtu.dk/fedora/objects/orbit:89486/datastreams/file_6341069/content (accessed Sept 9, 2013).
8. Fendrich, M., Johnson, T.P., Wislar, J.S., Hubbell, A. (2004) Drug test feasibility in a general population household survey. *Drug and Alcohol Dependence*, **73**, 237–250.
9. Fendrich, M., Johnson, T.P., Sudman, S., Wislar, J.S., Spiehler, V. (1999) Validity of drug use reporting in a high-risk community sample: a comparison of cocaine and heroin survey reports with hair tests. American *Journal of Epidemiology*, **149**, 955–962.
10. Samyn, N., Verstraete, A., van Haeren, C., Kintz, P. (1999) Analysis of drugs of abuse in saliva. *Forensic Science Reviews*, **11**, 2–17.
11. Wille, S.M.R., Raes, E., Lillsunde, P., Gunnar, T., Laloup, M., Samyn, N. *et al.* (2009) Relationship between oral fluid and blood concentrations of drugs of abuse in drivers suspected of DUID. *Therapeutic Drug Monitoring*, **31**, 511–519.
12. Langel, K., Gjerde, H., Favretto, D., Lillsunde, P., Øiestad, E.L., Ferrara, S.D. *et al.* (2013) Comparison of drug concentrations between whole blood and oral fluid. *Drug Testing and Analysis*, in press. doi:10.1002/dta.1532.
13. Gjerde, H., Langel, K., Favretto, D., Verstraete, A.G. (2013) Detection of 4 benzodiazepines in oral fluid as biomarker for presence in blood. *Therapeutic Drug Monitoring*, in press. doi:10.1097/FTD.0b013e3182a3ab42.
14. Gjerde, H., Verstraete, A. (2010) Can the prevalence of high blood drug concentrations in a population be estimated by analysing oral fluid? A study of tetrahydrocannabinol and amphetamine. *Forensic Science International*, **195**, 153–159.
15. Gjerde, H., Verstraete, A.G. (2011) Estimating equivalent cutoff thresholds for drugs in blood and oral fluid using prevalence regression: a study of tetrahydrocannabinol and amphetamine. *Forensic Science International*, **212**, e26–e30.
16. Pil, K., Raes, E., Verstraete, A. (2009) The toxicological challenges in the European research project DRUID. *Forensic Science International Supplement Series*, **1**, 29–32.
17. Gjerde, H., Christophersen, A.S., Normann, P.T., Mørland, J. (2013) Associations between substance use and fatal road traffic accidents among car and van drivers in Norway: a case-control study. *Transportation Research Part F: Traffic Psychology and Behaviour*, **17**, 134–145.
18. Assum, T., Mathijsen, M.P.M., Houwing, S., Buttress, S.C., Sexton, B., Tunbridge, R.J. *et al. The prevalence of drug driving and relative risk estimations. A study conducted in The Netherlands, Norway and the United Kingdom. IMMORTAL Deliverable D-R4.2.* Kuratorium für Verkehrssicherheit, Vienna, 2005.
19. Hels, T., Bernhoft, I.M., Lyckegaard, A., Houwing, S., Hagenzieker, M., Legrand, S.A. *et al. Risk of injury by driving with alcohol and other drugs. DRUID Deliverable D 2.3.5.* Technical University of Denmark: Copenhagen, Denmark, 2011. http://orbit.dtu.dk/fedora/objects/orbit:89488/datastreams/file_6341143/content (accessed Sept 9, 2013).
20. Lund, H.M., Øiestad, E.L., Gjerde, H., Christophersen, A.S. (2011) Drugs of abuse in oral fluid collected by two different sample kits—stability testing and validation using ultra performance tandem mass spectrometry analysis. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences*, **879**, 3367–3377.
21. van der Linden, T., Legrand, S.A., Silverans, P., Verstraete, A.G. (2012) DUID: Oral fluid and blood confirmation compared in Belgium. *Journal of Analytical Toxicology*, **36**, 418–421.
22. Vindenes, V., Lund, H.M., Andresen, W., Gjerde, H., Ikdahl, S.E., Christophersen, A.S. *et al.* (2012) Detection of drugs of abuse in simultaneously collected oral fluid, urine and blood from Norwegian drug drivers. *Forensic Science International*, **219**, 165–171.
23. Ferrara, S.D., Favretto, D., Montisci, M., Vogliardi, S., Stocchero, G., Viel, G. *et al.* (2011) Country report Italy. In Houwing, S., Hagenzieker, M., Mathijssen, R., Bernhoft, I.M., Hels, T., Janstrup, K. (eds). *Prevalence of Alcohol and Other Psychoactive Substances in Drivers in General Traffic. Part II: Country Reports. DRUID Deliverable D 2.2.3.* SWOV Institute for Road Safety Research, Leidschendam, The Netherlands, pp. 108–123. http://orbit.dtu.dk/fedora/objects/orbit:89805/datastreams/file_6418383/content (accessed Sept 9, 2013).
24. Ferrara, S.D., Favretto, D., Montisci, M., Vogliardi, S., Stocchero, G., Viel, G. *et al.* (2011) Country report Italy. In Isalberti, C., van der Linden, T., Legrand, S.A., Verstraete, A., Bernhoft, I.M., Hels, T. (eds.). *Prevalence of Alcohol and Other Psychoactive Substances in Injured and Killed Drivers. DRUID Deliverable 2.2.5.* University of Ghent, Ghent, Belgium, pp. 251–271. http://orbit.dtu.dk/fedora/objects/orbit:89487/datastreams/file_6341109/content (accessed Sept 9, 2013).
25. Langel, K., Gunnar, T., Ariniemi, K., Rajamaki, O., Lillsunde, P. (2011) A validated method for the detection and quantitation of 50 drugs of abuse and medicinal drugs in oral fluid by gas chromatography-mass spectrometry. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences*, **879**, 859–870.
26. Pehrsson, A., Gunnar, T., Engblom, C., Seppa, H., Jama, A., Lillsunde, P. (2008) Roadside oral fluid testing: comparison of the results of drugwipe 5 and drugwipe benzodiazepines on-site tests with laboratory confirmation results of oral fluid and whole blood. *Forensic Science International*, **175**, 140–148.
27. Walsh, J.M., Verstraete, A.G., Huestis, M.A., Mørland, J. (2008) Guidelines for research on drugged driving. *Addiction*, **103**, 1258–1268.
28. Simonoff, J.S. (2003) *Analyzing Categorical Data.* Springer Verlag, New York, NY.
29. Verstraete, A., Knoche, A., Jantos, R., Skopp, G., Gjerde, H., Vindenes, V. *et al.* (2011) *Per se limits—methods of defining cut-off values for*

zero tolerance. DRUID Deliverable D1.4.2. Ghent University, Ghent, Belgium. https://biblio.ugent.be/publication/1988464 (accessed Sept 9, 2013).
30. Simonsen, K.W., Steentoft, A., Hels, T., Bernhoft, I.M., Rasmussen, B.S., Linnet, K. (2012) Presence of psychoactive substances in oral fluid from randomly selected drivers in Denmark. *Forensic Science International*, **221**, 33–38.
31. Vindenes, V., Jordbru, D., Knapskog, A.B., Kvan, E., Mathisrud, G., Slørdal, L. *et al.* (2012) Impairment based legislative limits for driving under the influence of non-alcohol drugs in Norway. *Forensic Science International*, **219**, 1–11.
32. Smith, C., Sykes, A., Robinson, S., Thomas, E. (2011) Evaluation of blood microsampling techniques and sampling sites for the analysis of drugs by HPLC-MS. *Bioanalysis*, **3**, 145–156.