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Can the prevalence of high blood drug concentrations in a population be estimated by analysing oral fluid? A study of tetrahydrocannabinol and amphetamine.

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A B S T R A C T

Aim: To study several methods for estimating the prevalence of high blood concentrations of tetrahydrocannabinol and amphetamine in a population of drug users by analysing oral fluid (saliva).
Methods: Five methods were compared, including simple calculation procedures dividing the drug concentrations in oral fluid by average or median oral fluid/blood (OF/B) drug concentration ratios or linear regression coefficients, and more complex Monte Carlo simulations. Populations of 311 cannabis users and 197 amphetamine users from the Rosita-2 Project were studied.
Results: The results of a feasibility study suggested that the Monte Carlo simulations might give better accuracies than simple calculations if good data on OF/B ratios is available. If using only 20 randomly selected OF/B ratios, a Monte Carlo simulation gave the best accuracy but not the best precision. Dividing by the OF/B regression coefficient gave acceptable accuracy and precision, and was therefore the best method. None of the methods gave acceptable accuracy if the prevalence of high blood drug concentrations was less than 15%.
Conclusion: Dividing the drug concentration in oral fluid by the OF/B regression coefficient gave an acceptable estimation of high blood drug concentrations in a population, and may therefore give valuable additional information on possible drug impairment, e.g. in roadside surveys of drugs and driving. If good data on the distribution of OF/B ratios are available, a Monte Carlo simulation may give better accuracy.

Key-words: amphetamine; tetrahydrocannabinol; blood; oral fluid; prevalence; population; Monte Carlo simulation, linear regression

1. Introduction

Blood and urine are the most commonly used biological fluids for drug analysis. Urine sample analysis is used for the detection of drug use, while serum, plasma or whole blood analysis is required in cases of therapeutic drug monitoring, cases of suspected over-dosing, and when assessing possible drug impairment, e.g. among suspected drugged drivers. Oral fluid (mixed saliva) may also be analysed to detect and monitor drug use, and the use for this purpose is increasing [1-4]. Oral fluid is an easily available medium that can be collected with non-invasive methods without the intrusion of privacy. Oral fluid has about the same detection window (time range) as blood regarding alcohol and drugs [5]. A fairly small amount of oral fluid is needed for the analyses of alcohol and drugs [6], and the oral fluid sample may be collected within 2-5 minutes by using a simple, commercially available collection device [7].

Alcohol and most drugs enter oral fluid from blood by a passive diffusion process dependent on the compound's physicochemical properties, primarily pKa, protein binding, lipophilicity, molecular weight and spatial configuration [5]. Significant inter- and intra-subject variability in oral fluid/whole blood (OF/B) ratios has been observed [8-9], and the wide range of OF/B ratios does not allow reliable calculation of the drug concentrations in blood from drug concentrations in oral fluid. There are, however, positive correlations between drug concentrations in oral fluid and blood, varying from one drug to another [9-12]. For alcohol, the concentration in oral fluid reflects the blood alcohol concentration very well [1,4].

The technique used for sampling oral fluid may affect the analytical result. Physical or chemical stimulation of the production of oral fluid is often used in order to increase the sample volume; however, the concentration of drugs might in these cases be different from concentrations in non-stimulated oral fluid [8, 13-15]. The sampling device itself may also affect the analytical results because the recoveries of some drugs might vary from one device to another [7-8]. When comparing data from different studies on drug concentrations in oral fluid, it is therefore important to bear in mind the effect of different sampling procedures.

Oral fluid is often being collected in epidemiological studies of the prevalence of drug use, e.g. in roadside surveys of drug use among drivers. Our hypothesis is that the distribution of drug concentrations in oral fluid in a population of drug users is related to the distribution of drug concentrations in blood, and that the distribution of concentrations in oral fluid may be used to estimate the prevalence of blood drug concentrations above certain concentration limits. Such a method for utilising drug concentrations in oral fluid may give useful additional information in epidemiological studies.

A correlation between drug concentrations in oral fluid and blood was found for amphetamine, tetrahydrocannabinol (THC), and other drugs in the Rosita-2 study [9,16]. We examined the distribution of concentrations of amphetamine and THC from that study using the EasyFit software (www.mathwave.com) and found that two two-parameter probability distributions fitted the distributions of concentrations of both drugs in both blood and oral fluid well: the Weibull and Lognormal distributions. We also found that these probability distributions also fitted concentration data for zopiclone, diazepam, THC and codeine in samples of oral fluid from a study of random drivers [17]. We propose that if the oral fluid drug concentration distribution in a population fits a distribution model, a similar, plausible distribution in whole blood may be estimated. A number of representative OF/B ratios are needed for this estimation; to obtain OF/B ratios samples of oral fluid and blood must be obtained and analysed in the same way as for the study population.

The aim of the present study was to evaluate several methods for estimating the prevalence of high drug concentrations by calculating the accuracy (bias; deviation between estimated and observed prevalence of drug concentrations above chosen limits in blood) and precision (relative standard deviation, RSD) of the methods. Concentration data for THC and amphetamine observed in the Rosita-2 Project [9,16] were used to compare the estimation procedures.

2. Materials and methods

2.1. Study population

The Rosita-2 Project [9,16] included drivers who were apprehended by police in several countries suspected for driving under the influence of drugs. Samples of oral fluid were collected by using the Intercept Oral Specimen Collection Device (OraSure Technologies, Bethlehem PA, USA), and whole blood samples were also obtained. The samples were analysed for a number of psychoactive drugs. All drivers who tested positive for THC or amphetamine in samples of oral fluid, and from whom results for analysis of THC and amphetamine in blood were available, were selected for this study. Analytical findings have been presented elsewhere [9,16].

2.2. Analytical methods

Samples of whole blood and oral fluid were analysed by chromatographic-mass spectrometric methods. The drug concentrations in undiluted oral fluid were in some countries calculated by using the average dilution factor, in other countries by determining the dilution for each single sample by weighing the samples. References to different methods have been presented elsewhere [9].

2.3. Simple calculation methods for estimation of blood drug concentrations

A correlation between drug concentrations in oral fluid and blood has been found for both amphetamine and THC; for THC this correlation was more evident when examining the logarithmic values [9]. Therefore some simple methods were used to calculate virtual drug concentrations in blood based on actual concentrations in oral fluid, assuming that the distribution of the virtual blood drug concentrations would match the actual distribution of drug concentrations in blood.

Method A: Divide concentration in OF by average

The average OF/B ratio was calculated, and each drug concentration in oral fluid was divided by the average ratio giving a set of virtual blood drug concentrations. The prevalence of blood drug concentrations above or equal to a chosen concentration limit (e.g. THC $\geq$ 6.0 ng/ml) was estimated as the percentage of virtual blood drug concentrations above or equal to same limit. For the feasibility study all OF/B ratios were used to calculate the average, while for the validation studies 20 randomly selected OF/B ratios were used.

Method B: Divide concentration in OF by regression coefficient (slope)

The linear regression coefficient (slope) between oral fluid and blood was calculated using the "least squares" method by employing the LINEST function in Microsoft Excel with intercept at zero. Each drug concentration in oral fluid was divided by the regression coefficient giving a set of virtual blood drug concentrations that were used for calculations as described above.

Method C: Divide concentration in OF by median
The median OF/B ratio was calculated and used to calculate the prevalence and each drug concentration in oral fluid was divided by the median OF/B ratio giving a set of virtual blood drug concentrations that were used for calculations as described above.

2.4. Monte Carlo simulations

A Monte Carlo simulation is a method evaluating a deterministic model using sets of random numbers (or rather, pseudorandom numbers) as inputs, often iteratively [18-20]. Sets of random numbers complying with a chosen probability distribution function are generated, these numbers are included in more or less complex computations, and the outcome is evaluated mathematically.

If the distribution of blood drug concentrations in a population of drug users can be defined by a mathematical probability function, we expect that the distribution of drug concentrations in oral fluid can also be defined by a similar mathematical probability function, except that the parameters describing the distributions are different. The difference in the parameters are defined by the OF/B drug concentration ratios of that particular population.

If the blood drug concentration and OF/B ratio of each single individual in a population is known, the drug concentrations in oral fluid may be calculated by simply multiplying each blood drug concentration with the OF/B ratio. The distribution of drug concentrations in oral fluid may then be determined accurately. If the individual OF/B ratios are not known, the distribution of drug concentrations in oral fluid may be approximated by multiplying each single blood drug concentration with a random OF/B ratio from an equivalent population (later called “representative OF/B ratios” in this report). This approximation is used in the Monte Carlo simulation described below. This procedure is expected to give the best approximation if the OF/B ratio is independent of blood drug concentrations.

If we know the drug concentrations in oral fluid, and the individual OF/B ratios are not known, we cannot simply estimate the blood drug concentration by dividing the concentration in oral fluid by a random OF/B ratio; this procedure would generate an extremely wide distribution of virtual blood drug concentrations that would be very much different from the actual distribution. Therefore, a Monte Carlo simulation may be used to solve this problem. In our method, we generated a random distribution of simulated blood drug concentrations complying with a chosen probability distribution function (Lognormal or Weibull), then multiplied each simulated blood concentration with a random OF/B ratio to obtain simulated oral fluid drug concentrations. If the distribution of the simulated oral fluid drug concentration matched the actual distribution observed in the population being studied, the simulated blood drug concentration distribution was expected to describe a plausible distribution of blood drug concentrations. To obtain a match between the simulated and actual drug concentration distributions, the parameters describing the simulated blood drug concentration distribution were changed in an iterative process until a match was obtained.

The distributions that fitted the observed data for oral fluid and blood best were the Weibull and Lognormal distributions. It is easy to make calculations for the Lognormal probability distribution using a Microsoft Excel spreadsheet, whereas for the Weibull distributions it is more difficult without using so-called ad-ins for Excel. We have therefore described the use

of a spreadsheet performing Lognormal calculations below, and a modification using the Weibull probability distribution for drug concentrations in blood.

Method D: Monte Carlo simulation using Lognormal probability distribution
In the first step of our procedure, we calculated the actual lognormal parameters *meanlog* (M_{OF}) and *sdlog* (S_{OF}) for the observed distribution of drug concentrations in oral fluid: the *meanlog* is the mean of the natural logarithmic values of drug concentrations in oral fluid, and *sdlog* is the standard deviation of the logarithmic values.

An iterated Monte Carlo simulation was performed to determine a lognormal blood drug concentration distribution that would fit the observed oral fluid drug concentration. The simulation procedure is presented in Figure 1. Using a Microsoft Excel spreadsheet, 5000 random numbers (representing simulated blood drug concentrations) fitting a lognormal distribution with *meanlog* M_{Blood} and *sdlog* S_{Blood} were generated by using a combination of the inverse logarithmic function and the random function: LOGINV(RAND(),*meanlog*,*sdlog*) (use a semicolon instead of a comma as separator if your spreadsheet uses a decimal comma). As initial values, 1.00 was chosen for both M_{Blood} and S_{Blood}. Each of the 5000 random numbers was multiplied with one of the 20 selected OF/B ratios giving a simulated oral fluid drug concentration. The Lognormal distribution parameters *meanlog* M_{OFS} and *sdlog* S_{OFS} of the 5000 simulated oral fluid drug concentrations were calculated and compared with the *meanlog* M_{OF} and *sdlog* S_{OF} for the actual drug concentration distribution. In each of the following iteration steps of the procedure, the values for *meanlog* and *sdlog* for the blood concentration distribution (M_{Blood} and S_{Blood}) were slightly increased or decreased to obtain a converging fit between simulated and observed values for *meanlog* and *sdlog*. When the *meanlog* and *sdlog* for the simulated oral fluid drug concentrations (M_{OFS} and S_{OFS}) matched the actually observed *meanlog* and *sdlog* for the study population (M_{OF} and S_{OF}), with a maximum difference in *meanlog* and *sdlog* values of <0.01 ($|M_{OFS} - M_{OF}| < 0.01$ and $|S_{OFS} - S_{OF}| < 0.01$), the input values for *blood meanlog* and *blood sdlog* (M_{Blood} and S_{Blood}) used for the simulation were describing a plausible blood drug concentration distribution of the study population. The spreadsheet formulae and instructions for use are shown in Table 1.

< Insert Table 1 approximately here >
< Insert Figure 1 approximately here >

Method E: Monte Carlo simulation using the Weibull probability distribution
A Microsoft Excel spreadsheet can be used to generate random distributions fitting Weibull distributions, but cannot easily be used to calculate the distribution parameters *scale* and *shape* of an actual population. Since the Weibull and Lognormal distributions are similar, we used Lognormal calculations to compare the simulated and actual distributions of drug concentrations in oral fluid as a approximation, but generated blood drug concentration distributions complying with the Weibull distribution using the following formula in Microsoft Excel: c*(-LN(1-RAND()))^(1/m), where c = *scale* and m = *shape*. This formula was used in cells B7 to B5006 in the spreadsheet presented in Table 1, and s*cale* and *shape* replaced *meanlog* and *sdlog* in cells A3 and A4.

2.5. Feasibility study

An initial feasibility study was performed using the total population of subjects positive for amphetamine or THC in oral fluid, not sub-populations. For the simple calculation methods, the average and median of all OF/B ratios and the regression coefficient for the total number

of samples were used. For the Monte Carlo simulations, 20 OF/B ratios were systematically selected to represent the total distribution of OF/B ratios. For amphetamine, the 20 OF/B ratios were the 5th to the 98th percentile (i.e. percentiles 5.00, 9.89, 14.79, …, 93.11, 98.00). To exclude the most extreme ratios for THC, 20 OF/B ratios were selected evenly from the 8th to the 94th percentile.

2.6. Validation studies: determination of accuracy and precision

In most study situations, a limited number of representative OF/B ratios will be available for the actual oral fluid sampling method used and population studied. Therefore, a realistic procedure should be based on a reasonable number of OF/B ratios; we have used 20 ratios.

Twenty subjects with drug detected both in oral fluid and blood were selected at random from each of the populations of THC-positive and amphetamine-positive individuals by using the random function in a Microsoft Excel spreadsheet, and the OF/B drug concentration ratios were calculated. This selection of OF/B ratios was repeated five times, thus generating six sets of representative OF/B ratios for each drug in order to determine the precision of this method. The remaining subjects were used as study populations. The accuracy was calculated as average percent of the six sub-populations studied, while the precision was calculated as the relative standard deviation of the accuracies.

3. Results and Discussion

Tetrahydrocannabinol (THC) was detected in 311 samples of oral fluid from the Rosita-2 Project, and was thus the most commonly detected drug. Of the 311 persons who had THC concentrations above the analytical cut-off in oral fluid, 277 had also THC concentrations above the analytical cut-off in blood. Thus, altogether 277 OF/B ratios for THC were available. The OF/B ratios ranged from 0.006 to 569 with an average of 34.1 and a median value of 15.4, the apparent SD was 63.4. The distribution was thus very much skewed. The 10th and 90th percentiles corresponded to 0.1 and 5 times the median, respectively.

Amphetamine was the second most prevalent drug found in the Rosita-2 project. Altogether 197 subjects provided oral fluid samples that were positive for amphetamine, 187 of these subjects were also positive for amphetamine in blood. The OF/B ratios ranged from 0.27 to 210 with an average of 19 and a median of 12, the apparent SD was 26. The distribution was very much skewed, and the 10th and 90th percentiles corresponded to 0.2 and 3 times the median, respectively.

3.1. Feasibility study of the estimation methods

The prevalences of blood drug concentrations equal to or above specified limits determined in the feasibility study are presented in Tables 2 and 3.

When dividing the drug concentrations by the average OF/B ratio the obtained results matched the actual blood concentration data very well as far as the prevalence of blood amphetamine concentrations ≥800 and ≥1000 ng/ml was concerned. However, for THC the accuracy was poor, mainly due to the fact that the large number of very high OF/B ratios observed for THC gave a high average OF/B ratio. If using the average OF/B ratio for estimating the THC concentration distribution in blood, the highest OF/B ratios must therefore be excluded.

Dividing by the regression coefficient gave over-estimation of high drug concentrations for THC, but to a lesser extent for amphetamine. In total, using the regression coefficient seemed to give slightly more accurate estimations. Dividing by the median OF/B values gave poor accuracy for both THC and amphetamine and seems to be the least reliable method.

As far as Monte Carlo simulations were concerned, the Weibull simulation gave a better estimation of the actual drug concentration distributions in blood compared to the Lognormal simulation, but the results for the Lognormal simulation were also quite acceptable. The results suggest that good estimates may be obtained at optimal conditions, which may be obtained if large populations are studied, the Lognormal or Weibull distribution functions fit the drug concentration data in oral fluid, and a large number of representative OF/B ratios are available.

The Lognormal simulation gave a slight over-estimation of high drug concentrations in blood, while the Weibull simulation gave a slight under-estimation, probably because of the differences in the shapes of the Lognormal and Weibull probability distributions: a fitting Lognormal distribution has a larger right tail than a Weibull distribution, see Figure 2.

3.2. Validation studies using 20 random OF/B ratios

In most study situations, a limited number of representative OF/B ratios will be available for the actual oral fluid sampling method used and population studied. Therefore, a realistic procedure for the estimation of blood drug concentrations in a population should be based on a reasonable number of OF/B ratios; we decided to use 20 ratios for this study.

We chose 20 OF/B ratios at random from each population of drug users. In order to use independent data, the individuals providing the OF/B ratios that were used for the calculations were removed from the study population. We found that a random selection of 20 OF/B ratios in some cases contained several extremely high or extremely low values (< 0.1 times or > 5 times the median value), and therefore disabled the Monte Carlo simulation procedures (because the distribution of OF/B ratios was too large compared to the distribution of concentrations observed in oral fluid samples of the studied population). To eliminate this cause of error, we excluded OF/B ratios below 0.1 times the median and above 5 times the median of the selected OF/B ratios as outliers, and selected OF/B ratios until 20 ratios complying with this requirement were obtained. The validation results for THC and amphetamine for the Rosita-2 population are presented in Tables 4-5.

< Insert Table 4 approximately here >
< Insert Table 5 approximately here >

The precision and accuracy were not as good as for the initial feasibility study, primarily because that the estimations were based on a random selection of only 20 OF/B ratios which have a large variation. The RSD for the average OF/B ratios in the six selections were 14.0% and 32.3% for THC and amphetamine, respectively. The precision and accuracy for the estimation of blood amphetamine concentrations were worse than for THC, mainly because of larger variation in the OF/B ratios used for the calculations, but also because the population was smaller.

The accuracies for all estimation procedures were unacceptable for estimating the prevalence of THC concentrations ≥10.0 ng/ml (actual prevalence of 14.5%) and amphetamine concentrations ≥ 1000 ng/ml (actual prevalence of 11.9%). The precision RSD of all

estimation procedures were greater than 25% for most estimations of blood amphetamine concentrations, but less than or equal to 25% for all estimation procedures for THC.

For the simple calculation methods, best accuracy and precision was obtained when dividing the drug concentrations in oral fluid by the regression coefficient. For the Monte Carlo simulations, the Weibull simulation gave somewhat better accuracy but worse precision than the Lognormal simulation. In total, dividing by the regression coefficient seemed to be the best method for the populations of THC and amphetamine users in this study when the calculations were based on only 20 OF/B ratios. Better results are expected if a larger number of OF/B data are included in the calculations. We also expect that the methods would give better precision for drugs with more narrow distribution of OF/B ratios than THC and amphetamine.

4. Conclusion

The results suggest that the prevalence of blood drug concentrations above chosen limits may be roughly estimated by using analytical results for oral fluid when dividing drug concentrations in oral fluid by the OF/B regression coefficient or by using Monte Carlo simulations. Dividing by the regression coefficient gave better results than more advanced Monte Carlo simulations when only 20 OF/B ratios were available for the calculations. However, the results of the feasibility study suggested that Monte Carlo simulations may give better accuracy if OF/B ratios that are representative for the distribution in a larger population are available.

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Figures

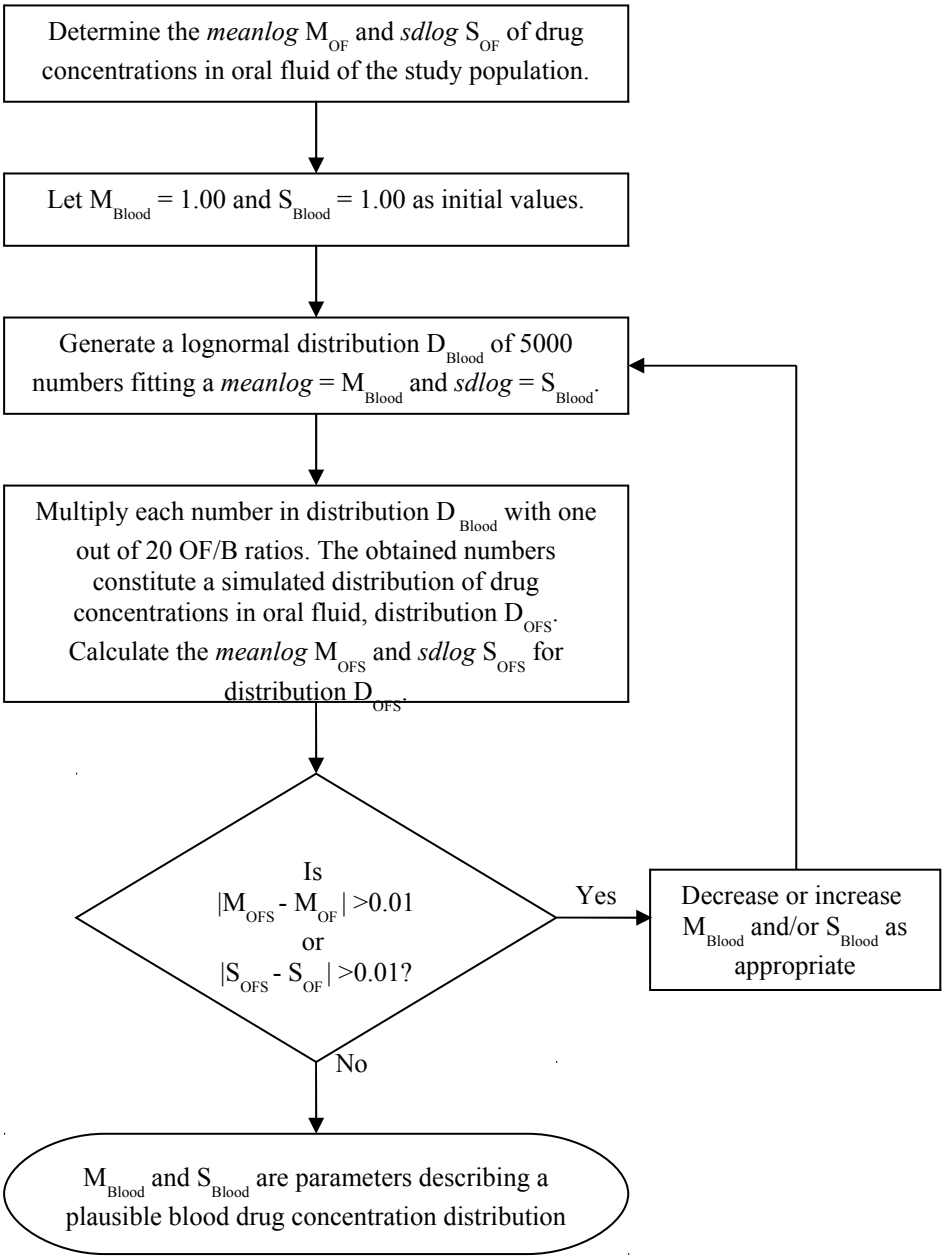

Figure 1. Flow diagram for the estimation of a plausible lognormal distribution of blood drug concentrations in a population based on drug concentrations in oral fluid. M_{OF} and S_{OF} are the *meanlog* and *sdlog* for the observed drug concentrations in oral fluid. M_{Blood} and S_{Blood} are the chosen *meanlog* and *sdlog* for drug concentrations in blood. M_{OFS} and S_{OFS} are the *meanlog* and *sdlog* for the simulated drug concentration in oral fluid calculated by using drug concentrations in blood.

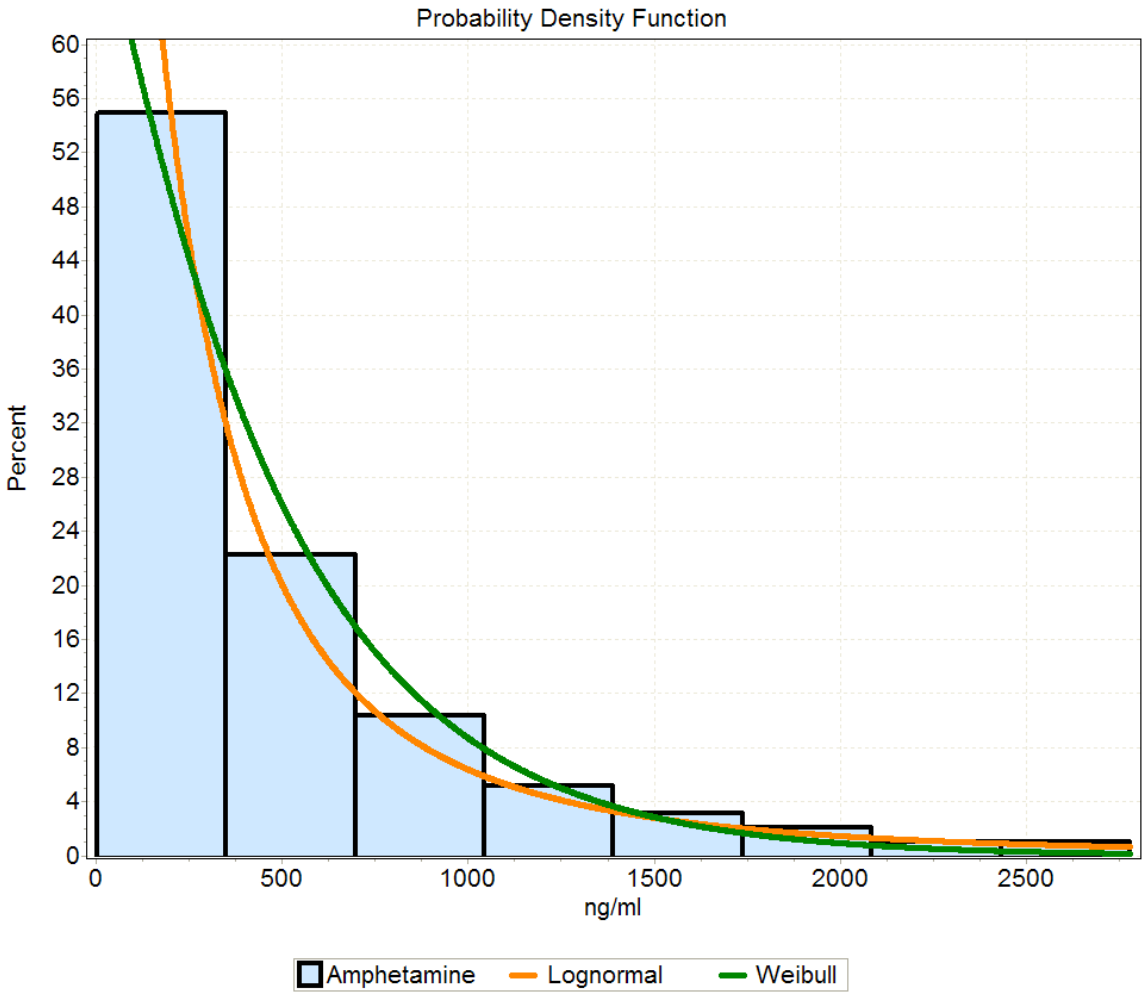

Figure 2. Observed distributions of amphetamine concentrations in blood in Rosita-2 Project and best fitted Lognormal and Weibull distributions.

Table 1. Microsoft Excel spreadsheet formulae (use a semicolon instead of a comma as separator if the spreadsheet uses decimal comma). Fill in representative 20 OF/B ratios in cells A7 to A26. Fill in the observed *meanlog* and *sdlog* for the drug concentration distribution of oral fluid in cells C3 and C4. Choose values for *blood meanlog* and *blood sdlog* to be entered into cells B3 and B4; initial values may be 1.00 for both parameters. Increase or decrease the numbers in B3 and B4 in a repeating process until the calculated values for D3 and D4 matches C3 and C4. Microsoft Excel recalculates the spreadsheet each time a number in B3 or B4 is changed or when the recalculate button is pushed.

	A	B	C	D
1		**Drug conc. distribution in blood**	**Drug concentration distribution in oral fluid**	
2		**Simulated**	**Observed**	**Simulated**
3	**Meanlog**			=AVERAGE(D7:D5006)
4	**SDlog**			=STDEV(D7:D5006)
5				
6	**OF/B ratios**	**Simulated drug concentrations in blood**	**Simulated drug concentrations in OF**	**LN(Simulated conc. OF)**
7		=LOGINV(RAND(),B3,B4)	=B7*A7	=LN(C7)
8		=LOGINV(RAND(),B3,B4)	=B8*A8	=LN(C8)
9		=LOGINV(RAND(),B3,B4)	=B9*A9	=LN(C9)
10		=LOGINV(RAND(),B3,B4)	=B10*A10	=LN(C10)
11		=LOGINV(RAND(),B3,B4)	=B11*A11	=LN(C11)
12		=LOGINV(RAND(),B3,B4)	=B12*A12	=LN(C12)
13		=LOGINV(RAND(),B3,B4)	=B13*A13	=LN(C13)
14		=LOGINV(RAND(),B3,B4)	=B14*A14	=LN(C14)
15		=LOGINV(RAND(),B3,B4)	=B15*A15	=LN(C15)
16		=LOGINV(RAND(),B3,B4)	=B16*A16	=LN(C16)
17		=LOGINV(RAND(),B3,B4)	=B17*A17	=LN(C17)
18		=LOGINV(RAND(),B3,B4)	=B18*A18	=LN(C18)
19		=LOGINV(RAND(),B3,B4)	=B19*A19	=LN(C19)
20		=LOGINV(RAND(),B3,B4)	=B20*A20	=LN(C20)
21		=LOGINV(RAND(),B3,B4)	=B21*A21	=LN(C21
22		=LOGINV(RAND(),B3,B4)	=B22*A22	=LN(C22)
23		=LOGINV(RAND(),B3,B4)	=B23*A23	=LN(C23)
24		=LOGINV(RAND(),B3,B4)	=B24*A24	=LN(C24)
25		=LOGINV(RAND(),B3,B4)	=B25*A25	=LN(C25)
26		=LOGINV(RAND(),B3,B4)	=B26*A26	=LN(C26)
27		=LOGINV(RAND(),B3,B4)	=B27*A7	=LN(C27)
28		=LOGINV(RAND(),B3,B4)	=B28*A8	=LN(C28)
…	…	…	…	…
5005		=LOGINV(RAND(),B3,B4)	=B5005*A25	=LN(C5005)
5006		=LOGINV(RAND(),B3,B4)	=B5006*A26	=LN(C5006)

Table 2. Determination of accuracy for the estimation of blood THC concentration distribution in the Rosita-2 Project population (N=311) using all or systematically selected OF/B ratios

		Method A: Divide concentration in OF by average		Method B: Divide concentration in OF by regression coefficient		Method C: Divide concentration in OF by median		Method D: Monte Carlo simulation, Lognormal distribution		Method E: Monte Carlo simulation, Weibull distribution	
Concentration (ng/ml)	Actual prevalence (%)	Estimated prevalence (%)	Accuracy (%)	Estimated prevalence (%)	Accuracy (%)	Estimated prevalence (%)	Accuracy (%)	Estimated prevalence (%)	Accuracy (%)	Estimated prevalence (%)	Accuracy (%)
≥ 2.0	59.5	37.6	63.2	46.3	77.8	52.1	87.6	53.0	89.1	59.6	100.2
≥ 4.0	41.2	25.4	61.7	33.4	81.3	39.2	95.3	35.1	85.4	39.7	96.6
≥ 6.0	28.0	17.0	60.9	26.0	93.1	31.8	113.8	26.2	93.5	28.6	102.3
≥ 8.0	20.9	12.2	58.5	18.0	86.2	24.4	116.9	20.2	96.5	20.5	98.3
≥ 10.0	14.1	8.7	61.4	17.0	120.5	23.2	163.6	16.3	115.4	14.7	103.6

Table 3.Determination of accuracy for the estimation of blood amphetamine concentration distribution in the Rosita-2 Project population (N=197) using all or systematically selected OF/B ratios

		Method A: Divide concentration in OF by average		Method B: Divide concentration in OF by regression coefficient		Method C: Divide concentration in OF by median		Method D: Monte Carlo simulation, Lognormal distribution		Method E: Monte Carlo simulation, Weibull distribution	
Concentration (ng/ml)	Actual prevalence (%)	Estimated prevalence (%)	Accuracy (%)	Estimated prevalence (%)	Accuracy (%)	Estimated prevalence (%)	Accuracy (%)	Estimated prevalence (%)	Accuracy (%)	Estimated prevalence (%)	Accuracy (%)
≥ 200	61.4	45.2	73.6	50.8	82.6	55.3	90.1	52.0	84.6	60.4	98.3
≥ 400	39.6	28.4	71.8	36.5	92.3	40.1	103.8	34.0	89.8	39.0	98.5
≥ 600	25.4	19.8	78.0	24.9	98.0	29.4	116.0	25.2	99.4	26.8	105.7
≥ 800	19.3	14.7	76.3	18.8	97.4	24.9	128.9	19.7	101.9	19.0	98.7
≥ 1000	12.2	10.2	83.3	14.7	120.8	19.3	158.3	16.1	132.8	13.3	109.2

Table 4. Determination of precision and accuracy (bias) for the estimation of blood THC concentration distribution in the Rosita-2 Project population (N=311).

		Method A: Divide concentration in OF by average			Method B: Divide concentration in OF by regression coefficient			Method C: Divide concentration in OF by median			Method D: Monte Carlo simulation, Lognormal distribution			Method E: Monte Carlo simulation, Weibull distribution		
Concentration (ng/ml)	Actual prevalence (%)	Estimated prevalence (%)	RSD (%)	Accuracy (%)	Estimated prevalence (%)	RSD (%)	Accuracy (%)	Estimated prevalence (%)	RSD (%)	Accuracy (%)	Estimated prevalence (%)	RSD (%)	Accuracy (%)	Estimated prevalence (%)	RSD (%)	Accuracy (%)
≥ 2.0	59.8	49.3*	6.1	82.4	48.7*	6.8	81.4	53.0*	7.6	88.6	54.1*	7.7	90.4	60.7	8.2	101.4
≥ 4.0	40.9	36.6*	6.5	89.6	35.5*	11.2	86.8	39.7	10.6	97.2	38.6	10.1	94.4	43.8	12.9	107.1
≥ 6.0	27.6	27.5	8.9	99.9	26.8	9.5	97.1	32.3*	14.1	117.6	30.0	13.6	108.8	33.1	19.5	120.0
≥ 8.0	20.5	23.5*	11.5	114.6	22.6	12.7	110.3	26.3*	11.4	128.6	24.6*	14.9	119.9	25.5*	24.2	124.1
≥ 10.0	14.5	19.4*	12.5	134.4	18.4*	13.8	127.1	23.3*	17.2	161.5	20.8*	14.2	143.8	20.2*	25.1	139.8

*The actual prevalence falls outside the estimated prevalence ± 1 SD.

Table 5. Determination of precision and accuracy (bias) for the estimation of blood amphetamine concentration distribution in the Rosita-2 Project population (N=197).

		Method A: Divide concentration in OF by average			Method B: Divide concentration in OF by regression coefficient			Method C: Divide concentration in OF by median			Method D: Monte Carlo simulation, Lognormal distribution			Method E: Monte Carlo simulation, Weibull distribution		
Concentration (ng/ml)	Actual prevalence (%)	Estimated prevalence (%)	RSD (%)	Accuracy (%)	Estimated prevalence (%)	RSD (%)	Accuracy (%)	Estimated prevalence (%)	RSD (%)	Accuracy (%)	Estimated prevalence (%)	RSD (%)	Accuracy (%)	Estimated prevalence (%)	RSD (%)	Accuracy (%)
≥ 200	60.9	50.7*	13.3	83.3	50.9*	14.2	83.6	55.7	12.6	91.4	52.7*	12.7	86.5	59.3	43.5	97.4
≥ 400	39.3	34.6	21.0	88.0	35.1	24.5	89.2	39.3	16.9	100.1	35.4	18.3	90.1	40.4	41.9	102.7
≥ 600	24.3	24.6	26.9	102.1	24.5	28.9	100.8	29.2	25.8	121.1	26.8	24.4	110.4	28.7	42.0	117.9
≥ 800	18.6	18.5	34.7	99.5	19.3	33.3	103.6	22.8	29.4	124.0	21.4	27.9	114.7	20.9	43.0	112.3
≥ 1000	11.9	15.4	34.7	129.6	15.3	30.9	128.9	18.0*	31.8	153.9	17.6*	29.4	148.0	15.5	47.6	130.5

*The actual prevalence falls outside the estimated prevalence ± 1 SD