The objective of this study was to compare the number of drivers with drug concentrations above the legal cutoffs for driving under the influence of illicit substances in paired samples of blood and oral fluid. Between January 2008 and September 2009, 2,949 randomly selected drivers participated in a roadside survey. Each was asked to provide blood and oral fluid. Samples were analyzed for 11 illicit substances or metabolites by ultra-performance liquid chromatography–tandem mass spectrometry and gas chromatography–tandem mass spectrometry. Out of the 2,750 drivers who gave both blood and oral fluid, 28 (1.0%) had drug concentrations above the legal cutoff in blood and 71 (2.5%) were above the legal cutoff in oral fluid. Fifteen (7.5%) of the 199 drivers who gave an oral fluid sample but refused to provide blood tested positive, significantly more than drivers who provided both samples. Based on oral fluid analysis, 2.6 times more subjects tested positive for drugs compared to blood analysis. Those that refused to give a blood sample were 3 times more likely to test positive for drugs. Even in a survey that guaranteed total anonymity, people fearing a positive test result might have been more likely to refuse to give a blood sample.

Introduction
The risks of driving after consuming alcohol are well known, unlike the dangers associated with using other psychoactive substances.

In 2009, a new law was passed in Belgium concerning the introduction of oral fluid on-site testing for drugs in traffic (1). Belgium is one of the first countries implementing a DUI law based on oral fluid for both screening and confirmation. In five Australian states, screening and confirmation are performed in oral fluid. This is different from other countries where confirmation analysis is performed on blood samples. In 2008, France passed a law with roadside oral fluid screening and confirmation in blood (2). Norway is planning to implement a law in 2012 based on oral fluid screening and blood confirmation. Since December 2010, Spain has also modified its law to allow oral fluid to be used as screening and in confirmation analysis, but no legal confirmation cutoffs have yet been established.

Previously in Belgium, a person suspected to be driving under the influence of an illegal substance mentioned in Belgian law had to undergo urine sampling, and confirmation analysis was performed on blood. If the roadside urine test was positive, the driver’s license was withheld for 12 hours.

In October 2010, the new law became effective. If the driver is suspected to be under the influence (verified by a standardized checklist) of one of the substances, an on-site oral fluid test is performed. When the result is at or above the cutoffs set in the law, a second oral fluid sample is taken for the confirmation analysis. If an oral fluid specimen cannot be collected, a blood sample is taken instead for confirmation purposes. Cutoffs for the on-site test and the confirmation analyses on both oral fluid and blood (plasma) are given in Table I.

Oral fluid offers many advantages as an alternative matrix for drug testing: sample collection is easy and non-invasive, which can be observed without the need of special restroom facilities and same-sex collectors. It also reduces the risk of adulteration and infection, and it better reflects recent drug use than urine sampling. The parent drug is prominent in oral fluid and provides some correlation with pharmacodynamic effects such as impaired performance (3, 4).

In oral fluid, ion trapping of basic drugs, such as amphetamine and cocaine, occurs because of pH differences between blood (7.4) and oral fluid (6.8 at rest). Free uncharged drug is in equilibrium between blood and oral fluid. At the lower pH in oral fluid, weak bases ionize, increasing total oral fluid drug concentrations. Because most narcotic drugs are basic, they are detected in higher concentrations in oral fluid than in plasma. The presence of THC in oral fluid comes largely from contamination and not portioning of blood to oral fluid (4, 5, 6, 7).

The objective of this article is to compare the number of drivers in Belgium who have drug concentrations above the legal cutoffs for driving under influence of illicit substances in blood and oral fluid, and in addition, to compare the percentage of positives in oral fluid for those who refused to give blood with the respondents who gave both sample types.

Methods
Between January 2008 and September 2009, a roadside survey was conducted in Belgium as part of the European integrated project DRUID (Driving Under the Influence of Drugs, Alcohol and Medicines). The objective of DRUID is to give scientific support to the European Union transport policy to reach the 2010 road safety target by establishing guidelines and measures to combat impaired driving. Geographic distribution of the roadside sessions was performed systematically: an equal number of sessions was scheduled in the catchment area of each hospital participating in a study of seriously injured drivers. (8, 9).

Each volunteer was asked to provide a blood sample (5-mL tube with potassium oxalate) and an oral fluid sample collected with the StatSure Saliva Sampler. The collection device consists of a cellulose pad on a plastic stick. When approximately 1 mL of sample has been collected, an indicator on the stick turns blue. The stick is then sealed in a tube containing 1 mL of buffer. Drivers could refuse to participate in the study, or if they wanted to participate, they could refuse to give a blood sample and only fill in a questionnaire and give an oral fluid
Table I
Cut-offs and blood/plasma ratios of substances recorded in the law

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>THC</td>
<td>25</td>
<td>15</td>
<td>10</td>
<td>1</td>
<td>0.55</td>
<td>16.25</td>
<td>2</td>
<td>1</td>
<td>10</td>
<td>27/1</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>50</td>
<td>50</td>
<td>25</td>
<td>25</td>
<td>0.65</td>
<td>16.25</td>
<td>50</td>
<td>20</td>
<td>25</td>
<td>360/20</td>
</tr>
<tr>
<td>MDMA</td>
<td>50</td>
<td>50</td>
<td>25</td>
<td>25</td>
<td>1.0*</td>
<td>25*</td>
<td>50</td>
<td>20</td>
<td>25</td>
<td>270/20</td>
</tr>
<tr>
<td>Morphine</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>1.02</td>
<td>10.2</td>
<td>40</td>
<td>10</td>
<td>25</td>
<td>95/10</td>
</tr>
<tr>
<td>6-acetylmorphine</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>0.57</td>
<td>5.7</td>
<td>4</td>
<td>10</td>
<td>10</td>
<td>16/10</td>
</tr>
<tr>
<td>Cocaine</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>25</td>
<td>1.0*</td>
<td>25*</td>
<td>8</td>
<td>10</td>
<td>25</td>
<td>170/10</td>
</tr>
<tr>
<td>Benzoylcegonine</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>25</td>
<td>1.0*</td>
<td>25*</td>
<td>8</td>
<td>50</td>
<td>25</td>
<td>95/50</td>
</tr>
</tbody>
</table>

* no ratio available in the literature, presumed to be 1.

A total of 2,750 drivers provided both a blood and an oral fluid sample, while 199 drivers only provided an oral fluid sample.

Samples were transported under cooled conditions to the laboratory where the toxicological analyses for 11 illicit psychoactive substances and metabolites were performed.

In oral fluid, substances were analyzed using liquid–liquid extraction (LLE) followed by ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS-MS) (10). Tetrahydrocannabinol (THC) in blood samples was initially screened for using enzyme-linked immunoassay (ELISA) and confirmed using LLE followed by gas chromatography–mass spectrometry (GC–MS) (11). Solid-phase extraction of blood samples followed by UPLC–MS-MS was used to analyze for the other illicit substances (9).

The amount of collected oral fluid was determined by weighing the collector. The dilution with buffer was taken into account when calculating analytical results expressed in g/L or ng/mL of undiluted oral fluid. This was calculated using the following formula:

\[
C_{\text{corrected}} = \frac{C_{\text{uncorrected}}x(1 + w - \overline{w})}{2x(w - \overline{w})} \tag{1}
\]

where \(\overline{w}\) = average weight of empty StatSure device \(w\) = weight of sample and StatSure device \(C_{\text{uncorrected}}\) = uncorrected concentration of analyte \(C_{\text{corrected}}\) = concentration of analyte corrected for volume of oral fluid collected.

The results of analysis were interpreted as positive or negative based on the confirmation cutoffs for oral fluid and plasma in the Belgian law (Table I) (1).

As part of the uniform methods used in the DRUID epidemiological studies, whole blood was analyzed (12). A blood/plasma ratio was used to convert the Belgian legal plasma cutoffs into whole blood values. If no ratio was available from the literature, it was presumed to be 1 (Table I) (13).

Percentages of positive findings and concentration ranges were calculated using Microsoft Office Excel 2007. Chi-square and Fisher Exact were calculated with the MedCalc software (Mariakerke, Belgium) and used to determine differences in distribution.

The study protocols were approved by the ethics committee of Ghent University Hospital. The standard protocol complied with recognized standards of human subjects' protection.

Results

Out of the 2,750 respondents for which blood and oral fluid samples were available, 39 blood concentrations and 106 oral fluid concentrations were above the cutoff for illicit substances mentioned in the Belgian law (Figure 1). Taking into account the number of respondents positive for more than one illicit substance, 28 (1.0%) respondents would be sanctioned for driving under the influence of illicit drugs according to the cutoff in blood; 71 (2.6%) respondents would be sanctioned according to the cutoff in oral fluid. This difference is significant (\(P < 0.01\)).

Figure 1 shows the concentrations of the drugs in blood and oral fluid. A higher number of positives in oral fluid compared to blood was observed for all drugs. The number of positive samples in blood and oral fluid, respectively, was 2 and 0 for amphetamine, 25 and 7 for cocaine, 24 and 12 for benzoylcegonine, 40 and 13 for THC, 12 and 5 for morphine and 3 and 2 for 6-acetylmorphine. The average ratio of positive in oral fluid to positive in blood was 2.5, ranging from 1.5 for 6-acetylmorphine to 3.6 for cocaine.

A second comparison was made in addition. The results on positive oral fluid samples in the group giving both samples was compared to the positive results in the group of those who did not give a blood sample.

Out of the 199 respondents for which only an oral fluid sample was available, 26 (13%) oral fluid concentrations were above the cutoff for illicit substances mentioned in the Belgian law (Table II). Taking into account the number of respondents positive for more than one illicit substance, 15 respondents (7.5%) would be punishable for driving under the influence of illicit drugs. This percentage was significantly (\(P = 0.0005\)) higher than in the group that also gave a blood sample (2.6%).

No significant difference was found between both groups concerning the gender distribution (\(P = 0.447\)); in both groups, males were more represented than females. Concerning age, no difference in mean was found between both groups (\(P = 0.242\)), however, when dividing into age groups, statistical differences were found for the groups 18–24 y (\(P = 0.0373\)) and 35–49 y (\(P = 0.0352\)). Drivers aged 18 till 24 were found more in the...
The figures show that the Belgian law is stricter now that confirmation is performed on oral fluid samples rather than in blood. The group of respondents who refused to give a blood sample turned out to have a higher number of positives. People driving under influence of drugs were probably more reluctant to provide a blood sample in a situation when driving under the influence of drugs can be sanctioned based on the analysis of blood.

Most oral fluid/blood ratios implicate higher concentrations of drugs in oral fluid. Comparing both sets of cutoffs, therefore, it is remarkable that the limits in oral fluid are equal to or lower than those in blood (except for THC). Nevertheless, the values are in line with recommended oral fluid cutoffs by SAMHSA (Substance Abuse and Mental Health Service Administration), DRUID and the expert group that met at Talloires (4, 18, 19, 20).

Table II gives an overview of the percentage of drivers positive for the different substances included in the Belgian law within each group, together with the \( p \)-values of the Fisher exact tests. Statistically significant differences were observed for THC, 6-acetylmorphine, cocaine and benzoylcegonine.

### Comparison of the percentage of positives in oral fluid above the cutoffs in the Belgian Law between the group of drivers who gave only an oral fluid sample and the group who gave both blood and oral fluid samples

<table>
<thead>
<tr>
<th>Substance</th>
<th>Percentage of positives</th>
<th>( p ) (Fisher exact test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Only oral fluid sample</td>
<td>Oral fluid and blood sample</td>
</tr>
<tr>
<td></td>
<td>available (n= 199)</td>
<td>available (n = 2750)</td>
</tr>
<tr>
<td>THC</td>
<td>3.52</td>
<td>1.45</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>0.07</td>
<td>0</td>
</tr>
<tr>
<td>MDMA</td>
<td>0.50</td>
<td>0</td>
</tr>
<tr>
<td>Morphine</td>
<td>1.51</td>
<td>0.44</td>
</tr>
<tr>
<td>6-acetylmorphine</td>
<td>1.01</td>
<td>0.11</td>
</tr>
<tr>
<td>Cocaine</td>
<td>3.52</td>
<td>0.91</td>
</tr>
<tr>
<td>Benzoylcegonine</td>
<td>3.02</td>
<td>0.91</td>
</tr>
</tbody>
</table>

THC: \( \Delta^1 \)-tetrahydrocannabinol
* significant difference

group of drivers who gave only an oral fluid sample, the opposite was observed for drivers aged 35–49.

Table II gives an overview of the percentage of drivers positive for the different substances included in the Belgian law within each group, together with the \( p \)-values of the Fisher exact tests. Statistically significant differences were observed for THC, 6-acetylmorphine, cocaine and benzoylcegonine.

### Discussion

When using the cutoffs of the Belgian law, 1.0% of the driving population would be punishable for driving under the influence of illicit drugs based on the analysis of blood, and 2.9% would be punished based on oral fluid analysis. Recent studies in Europe and Australia have shown similar percentages of drivers testing positive for illicit substances. On a European level, illicit drugs are estimated to be used by 1.90% of drivers (14). Weighted prevalence for amphetamines, cannabis and cocaine was 0.1, 1.3 and 0.4%, respectively (based on oral fluid and blood results, using equivalent cutoffs; Table I). In Victoria, 2.4% of screened drivers tested positive for cannabis, MDMA or amphetamines (15). Two studies conducted in Queensland in 2007 and 2009 reported that 3.5 and 3.7%, respectively, of the included drivers were confirmed positive for at least one illicit substance (16, 17).

The figures show that the Belgian law is stricter now that confirmation is performed on oral fluid samples rather than in blood. The group of respondents who refused to give a blood sample turned out to have a higher number of positives. People driving under influence of drugs were probably more reluctant to provide a blood sample in a situation when driving under the influence of drugs can be sanctioned based on the analysis of blood.

Most oral fluid/blood ratios implicate higher concentrations of drugs in oral fluid. Comparing both sets of cutoffs, therefore, it is remarkable that the limits in oral fluid are equal to or lower than those in blood (except for THC). Nevertheless, the values are in line with recommended oral fluid cutoffs by SAMHSA (Substance Abuse and Mental Health Service Administration), DRUID and the expert group that met at Talloires (4, 18, 19, 20).

The legal confirmation cutoffs for oral fluid have been determined in a function of the screening cutoffs. The latter were based on the detection limits of existing on-site tests. The screening cutoffs are similar to, or somewhat higher than, those in France (Table I), and also higher than those used in Victoria (2 ng/mL for THC, and 5 ng/mL for methamphetamine and MDMA) (15). The confirmation cutoffs were set at half the screening values. If confirmation cutoffs in oral fluid would be set equivalent to blood (i.e., higher values in oral fluid as stated in the current law), a significant number of false positive screenings would occur, with consequences such as 12-hour withdrawal of the driving license. In addition, the law is a zero-tolerance law. Still, some criticism was raised about this legislation, primarily because a substance can still be identified days after consumption, when it is no longer producing psychotropic effects. According to critics, this is no longer related to road safety issues (21, 22). However, the previous legislation included screening in urine, with a much longer detection window, that resulted in approximately 15% false-positives (positive urine screenings that could not be confirmed in blood) (23).

### Conclusion

Depending on the cutoffs, using oral fluid as matrix could increase the number of positives in roadside surveys and enforcement of drugged driving legislation. This will result in detecting more people who drive after consumption of an illicit substance. Many will argue that at the current low cutoffs in oral fluid, the drivers are not under the influence, although for example, there is evidence that many accidents occur in the “crash” phase long after taking stimulant drugs (24). In many European countries and US states, there is a zero tolerance for drugs in traffic. Lower cutoffs in oral fluid might result in more apprehensions.

### Acknowledgments

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Disclaimer

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References