Expert Panel Review of alternative biological matrices for use as an evidential sample for drug driving
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Expert Panel Review of alternative biological matrices for use as an evidential sample for drug driving

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EXECUTIVE SUMMARY

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1 Methodology and Analytical Techniques Available for the Detection of Drugs Included in the Drug-Driving Legislation

It is standard practice in Forensic Science for the methodology employed for the screening test to be qualitatively different from the confirmation test. In the UK drug-driving context, a Point of Contact Test (POCT) for an oral fluid (OF) screening device is used as the initial test and whole blood for the confirmatory (evidential) test. The confirmatory test is carried out in the laboratory using liquid or gas chromatography and mass spectrometry (LC or GC-MS). It is a requirement of law that confirmatory (evidential) tests are undertaken.

1.1 Screening Tests
Immunosassays are the basis for initial screening tests (POCT) undertaken at the roadside or in the police station. Such testing offers advantages in simplicity and ease of performance but this may be offset by potential problems concerning the lack of a positive control. Those interpreting the test result to reach a conclusion with serious consequences such as driving under the influence of drugs need to be sure of the validity of the findings. Drug testing at the roadside can therefore only be carried out with equipment that has been type-approved by the Secretary of State after testing by the Home Office’s Centre for Applied Science and Technology (CAST).

Although roadside screening to detect psychoactive drug use is carried out using an OF immunoassay, developments such as the portable Surface-Enhanced Raman Spectroscope (SERS) analyser may offer an alternative technique, particularly if a handheld version comes to market.

In terms of roadside testing the Panel recommends that the Home Office could expand the list of type-approved screening tests to include, in addition to THC and cocaine (which also provides a route to the cocaine metabolite benzoylcegonine (BZE)), the amphetamine-type drugs (methamphetamine and MDMA) and ketamine to reflect the growing use of these compounds in driving populations.

1.2 Confirmatory Tests
More sophisticated equipment is needed for the confirmatory test than the screening test. Sample preparation (the extraction of the drug from the biological matrix) is required in order to quantify the presence of a specific drug. The direct injection of neat biological sample into chromatographic systems is an emerging development and significantly speeds up the analytical process.
The confirmatory test can only take place in an accredited Forensic Service Provider (FSP) laboratory. The Panel confirmed that the substances included in section 5A of the drug-driving legislation can be easily quantified in whole blood or OF in the laboratory using GC or LC-MS.

1.2.1 Relationship between whole blood, Oral Fluid (OF), urinary, and hair drug concentrations

It is widely accepted that blood and, to a lesser degree, OF are likely to give the most accurate measurement of drugs currently active in the body. Urine provides a somewhat longer detection period (drug use over the last 2-3 days), but with less quantitative accuracy. Hair provides a substantially longer detection window but does not usually used to indicate recent drug use in relation to a road traffic incident.

The Panel acknowledged that OF collected at the roadside for confirmatory purposes would be particularly advantageous in terms of reducing the time lag between the driving incident and evidential testing for illicit drugs such as THC, cocaine and heroin that are known to decline rapidly in blood after use.

1.2.2 The maturity of confirmatory analysis in blood and OF

To date, OF tests have not been fully validated as an alternative tool to whole blood for ‘per se’ evidential testing but, has more potential when a Lowest-Limit-of-Quantification (LLOQ) approach is utilised. The scientific evidence suggests that commercial OF immunoassay POCT devices are not suitable for evidential testing (although some devices allow for the collection of OF for separate evidential testing at the laboratory).

The Panel recognised maturity in the chromatographic methodology available for the analysis of drugs included in the section 5A offence and this was at the level of sophistication that enabled the measurement of low concentrations of drugs in various matrices including whole blood and OF.

The Panel recommends that the validity of the evidential drug-test requires that the level of confidence in the analytical methodology be known. Uncertainty data following chromatographic analysis of different drugs in whole blood (summarised in Table 1), can be found in the literature and are presented in the full report. The Panel noted that for
whole blood an analytical uncertainty based on 3 standard deviations \((k = 3)\)\(^1,2\) was preferred by the Home Office and should be achievable for chromatographic assays (confirmatory tests) of the drugs included in the Section 5A offence as follows:

Table 1  Uncertainty data following chromatographic analysis of different drugs in whole blood summarised from the scientific literature (section 2.7 full report)

<table>
<thead>
<tr>
<th>Drug</th>
<th>% Uncertainty</th>
<th>Drug</th>
<th>% Uncertainty</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC</td>
<td>16 – 30.0</td>
<td>Amphetamine</td>
<td>34</td>
</tr>
<tr>
<td>Cocaine</td>
<td>21 - 29.5</td>
<td>Morphine</td>
<td>33 – 45</td>
</tr>
<tr>
<td>BZE</td>
<td>17 - 30.5</td>
<td>Methadone</td>
<td>27-33</td>
</tr>
<tr>
<td>LSD</td>
<td>30</td>
<td>Diazepam</td>
<td>7-12</td>
</tr>
<tr>
<td>MDMA</td>
<td>24</td>
<td>Lorazepam</td>
<td>&lt;33</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>&lt;30</td>
<td>Oxazepam</td>
<td>&lt;30</td>
</tr>
<tr>
<td>6-MAM</td>
<td>42</td>
<td>Temazepam</td>
<td>&lt;30</td>
</tr>
<tr>
<td>Ketamine</td>
<td>35</td>
<td>Clonazepam</td>
<td>&lt;34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flunitrazepam</td>
<td>&lt;36</td>
</tr>
</tbody>
</table>

2 IDENTIFICATION OF ALTERNATIVE BIOLOGICAL MATRICES FOR EVIDENTIAL TESTING OF DRIVERS APPREHENDED FOR DRUG DRIVING

2.1 BLOOD

Whole blood is currently the matrix used for evidential testing in those suspected of committing a drug-driving offence in Great Britain. This is based on the sound principle that drug concentrations in blood provide an accurate picture of the amount of drug(s) present in the body at the time of sampling and presents the strongest scientific evidence in relation to impaired driving performance. It remains the gold standard in this regard.

However, the Panel acknowledged that care needs to be taken when collecting blood to ensure that sample integrity is assured; appropriate use of a preservative and an

\(^{1}\) For a confidence level of 99.7 % with a normal distribution. Measurement Good Practice Guide No. 36, National Physics Laboratory, 2004; G104 - Guide for Estimation of Measurement Uncertainty In Testing, American Association for Laboratory Accreditation, December 2014

\(^{2}\) It is worth noting that the test being applied is whether a measured value is greater than the legal limit. This is a one-sided statistical test and hence the probability needs to be halved, i.e. 99.7 % (0.3 %) becomes 99.85 % (0.15 %) if you use \(k=3\) for the confidence interval.
anticoagulant is mandated. Standardisation of the sampling kit and blood collection tubes for evidential tests are warranted with attention given to temperature and light during storage, transportation, and the timeliness of sample collection in relation to the driving incident. The Panel recommends that where whole blood is used for evidential tests there should be a specification (minimum standard) for the sample collection kit and the blood collection tube that includes details of the amount of preservative and anticoagulant required. The Panel also recommends moving towards the use of a vacutainer blood sampling device for safer sampling and in keeping with current practice within the NHS.

2.2 **Urine**

Urine reflects drug use over the previous few days or longer and in this sense is not helpful in the drug-driving context since a relationship between observed impairment or time when drug consumption last occurred cannot be determined. Despite the advantage of having a matrix that requires little laboratory preparation and that can be collected in large volumes, urinalysis presents difficulties as a confirmatory test for drug driving purposes when efforts are made to correlate drug concentration directly with impairment. Urine drug test results provide information regarding the manner in which a drug is eliminated from the body rather than an indication of drug activity in the body. Excretion patterns of drugs vary within and between individuals such that the setting of cut-off values with this in mind would be very difficult. Although the relationship between blood concentrations and urine concentrations have been researched over many years, the general consensus is that urine cannot be used to determine current pharmacological drug activity in the body.

The other major limitation with urine testing is with regard to the inconvenience of urine sample collection and the potential lack of integrity. Unless voidance of urine is observed, the authenticity of the sample may be called into question since urine can easily be contaminated. The possibility of false negative results following adulteration of urine with chemicals or by dilution is well described. Special facilities must be provided to be able to observe the sample collection to avoid adulteration, which may be time consuming and impractical. Urine is only suitable for the confirmation that drug use has taken place at some point in the past.

However, when a zero tolerance approach is used and a laboratory LLOQ analysis is employed as the cut-off, then urinalysis can be used to support an impairment test result, as is the case for section 4 legislation.
2.3 ORAL FLUID (OF)

There are several potential advantages with OF not least that it is readily accessible, and requires no medical personnel for sampling. OF collected at the roadside for confirmatory purposes would remove the need for specialist personnel to attend the police station to carry out sampling and therefore reduce the delay between the driving incident and sample collection so that drug concentrations reflective of those at the time of the driving incident were more likely.

A threshold-based approach where the intention is to detect the presence of a psychoactive substance using OF and relate it to a time when the driver had been apprehended deemed impaired and to be a high road safety risk would be problematic. For confirmatory testing purposes using a per se (threshold) approach, the usefulness of OF as a possible matrix would be dependent on consistent oral fluid-whole blood (OF: B) ratios. For OF concentrations to predict whole blood concentrations accurately, the OF: B ratio would need to be independent of drug concentration and consistent within and between individuals.

However due to large individual variations, ratios have been difficult to agree and cannot be easily determined for most psychoactive drugs, although some correlation has been described. OF: blood ratios have been shown to vary from drug to drug, from person to person, and even intra-individually making efforts to relate OF drug concentration at an equivalent blood drug concentration very challenging. The Panel agreed that blood concentrations could not be correlated with those in OF so that the ‘risk threshold’ limits set in the Section 5A legislation for medicinal controlled drugs (UK Government, 2014) could not be translated into OF cut-offs.

The Panel concluded that the wide range of the ratios recorded does not allow reliable estimation of blood drug concentrations from OF concentrations. OF could conceivably be utilised for evidential testing where a LLOQ approach was employed and in this regard OF testing would be much better suited to illicit substances than to medicinal controlled drugs.

For evidential testing using OF, the Panel recommended the OF cut-off limits published in the ‘Guide to Type-Approval Procedures for Preliminary Drug Testing Devices Used for Transport Law Enforcement in Great Britain’ in 2012, could be used with illicit drugs (Table 2). These were based on the approach taken for whole blood, i.e. the lowest limit of

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3 http://www.gov.uk/government/collections/drug-driving/
quantification (LLOQ) that most laboratories would be able to detect, yet above the concentrations commonly associated with passive (accidental) exposure.

Table 2  **Lowest Limit of Quantification (Cut-offs) for illicit drugs detected (µg/L) in OF (above concentrations commonly associated with passive exposure).**

<table>
<thead>
<tr>
<th>Drug Group</th>
<th>Drug to be detected</th>
<th>LLOQ Cut-off (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannabinoids</td>
<td>Delta-9-THC</td>
<td>10</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Cocaine</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Benzoylecgonine</td>
<td>30 (as composite)</td>
</tr>
<tr>
<td>Amphetamines*</td>
<td>Methamphetamine</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>MDMA</td>
<td>25</td>
</tr>
<tr>
<td>Opiates (heroin)</td>
<td>6-MAM</td>
<td>10</td>
</tr>
<tr>
<td>Hallucinogens</td>
<td>LSD</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Ketamine</td>
<td>20</td>
</tr>
</tbody>
</table>

*The spelling of amphetamine is in keeping with the Misuse of Drugs Act 1971 rather than the International Nonproprietary name (INN), amfetamine.*

This matrix has been successfully used for preliminary drug testing (screening) where immediate results are required and, to this end, a number of jurisdictions around the world have adopted OF as a roadside screening tool for the detection of illicit drugs and psychoactive medicines in those suspected of drug-driving offences. However, the scientific evidence suggests that commercial OF immunoassay POCT devices are not suitable for evidential testing (indeed they were not designed with this in mind). This should not defer efforts to explore this matrix using other methodology.

Although many OF immunoassay POCT drug screening devices involve the collection of a small volume of OF, some have a facility to send part of the sample to the laboratory for evidential tests. Evidential tests using the OF Cozart® Rapiscan has been successfully used in the State of Victoria, Australia for the detection of illicit substances (MDMA, THC and methamphetamine) in apprehended drug drivers.

There would be practical limitations to overcome in order to use POCT OF devices for evidential testing. Should such an approach be envisaged and given the variability in performance of the commercial OF POCT devices on the market, the Panel recommend
that sensitivity, specificity and accuracy criteria should be specified for OF POCT device(s). Criteria used for evidential tests has been set at ≥95 % (accuracy), ≥90 % sensitivity and ≥90 % specificity in the State of Victoria, Australia. Minimum standards were also established by the European Integrated Project DRUID (Driving under the Influence of Drugs, Alcohol and Medicines)\textsuperscript{4} and was set at 80% for each parameter; the Dräger DrugTest (DDT) 5000\textsuperscript{5} type-approved by the Home Office fulfilled the DRUID criteria for all drug classes included in the section 5A offence.

Evidential testing to quantify up to 17 compounds would likely require significantly more OF than currently used in the POCT roadside screening test: estimates by FSPs suggest 2-4 mL OF would be required. The collection of OF would therefore need to involve an OF collection device. There are important differences between OF commercial collection devices currently on the market. Buffer solutions are varied and differing volumes of OF/buffer solutions are collected which require ‘correction’ before reporting quantitative results.

If OF is to be collected using a commercial device for evidential purposes, then the Panel recommend that recovery of the analytes of interest and the overall reliability of the device would need to be specified for use at the roadside or in the police station. The collection of the OF needs to be used in conjunction with collection volume imprecision data (i.e. whether the OF collected was above, below or had achieved the minimum volume required), and uncertainty of measurement to provide the OF drug concentration in neat fluid to satisfy the criminal justice system. The specification (minimum standard) for the sample collection kit and the OF collection tube should include details of the amount of preservative, stabiliser and buffer required. Whilst full type-approval is unlikely to be necessary the equipment would need to be independently assessed so that it meets the above standards. This might be part of the FSP accreditation process (See Section 4 on page 11).

Based on what is known, the Panel recommends that OF samples should be refrigerated (3 – 5 °C) as quickly as possible after collection and transported to the laboratory at a controlled temperature to avoid bacterial contamination and degradation of drugs. It is also recommended that OF samples should be stored in glass tubes, away from fluorescent light and direct sunlight. OF samples should be frozen (ideally at -20 °C), if not available for immediate analysis.

\textsuperscript{4} DRUID Project co-funded by the European Commission within the Sixth Framework Programme (2002-2006), Project No: TREN-05-FP6TR-S07.61320-518404-Deliverable (0.1.8), Final Report: Main results and recommendations (2012). www.druid-project.eu/
OF as a possible matrix for confirmatory testing may have other limitations. Potential confounders include the effects of pH variation on the appearance of drugs in OF, the potential for buccal cavity contamination and dry mouth syndrome (hyposalivation).

To conclude, there is a stronger argument for the use of OF as an evidential matrix when using laboratory based cut-offs (LLOQ) such as those suggested by the DRUID studies, as the concentration above which an offence would occur. This approach would be in line with a zero tolerance approach, rather than a road safety risk based approach. With regards to the drug cut-off levels in the section 5A regulations, OF limits could not be identified for the medicinal drugs where a risk-based approach underpins the cut-off concentrations in whole blood.

2.4 SWEAT

The use of a sweat POCT screening device has been employed in Europe to test those apprehended at the roadside and thought to be under the influence of illicit drugs. This approach uses immunoassay devices also employed for OF collection. However, sweat testing per se has yet to be shown to be applicable for confirmatory drug-driving tests. Consideration would need to be given to the issue of external contamination and how this can be negated as part of the sample collection procedure.

2.5 HAIR

It takes about 7 days for a drug to be incorporated into a hair follicle making any attempt to correlate hair drug concentration with driving behaviour extremely difficult. This factor and the inconvenience of sample collection and the requirement for enough hair sample to be collected to test for all 17 compounds in the schedule 5A legislation impact on the usefulness of hair as an evidential test matrix. However, hair testing has been used in many European countries to confirm abstinence from illicit drugs in persons whose driving licences have been suspended for drug-driving offences. The Panel recommends that hair testing is an appropriate matrix to use as an adjunct to medical assessment for re-licensing since hair testing provides a much longer window of detection than either blood, urine or OF and would enable the determination of a history of past exposures to illicit or medicinal controlled substances.

2.6 DRIED BLOOD SPOTS (DBS) AND SMALL LIQUID SAMPLES

As yet the procedures and technology for either dried blood spot (DBS) sampling or small liquid samples has not progressed far enough to be used as an evidential test in an environment such as a police station or at the roadside. However, the development of
commercial devices may be suitable in the future for use by law enforcement officers in those suspected of drug-driving offences.

2.7 **LATENT FINGERPRINTS**

The analysis of drugs in latent fingerprints is an exciting new development that shows promise in a number of arenas that require flexible drug screening services. The Panel noted that quantitative analysis of drugs of interest is not currently well developed and therefore could not recommend the use of latent fingerprints as an alternative to blood for evidential testing. Consideration will need to be given to the issue of external contamination and how this can be negated as part of the sample collection procedure.

2.8 **EXHALED BREATH CONDENSATE (EBC)**

Exhaled breath condensate (EBC) is a further innovation with regard to drug testing matrices and is based on the premise that therapeutic and illicit drugs are present as non-volatile components in human breath. In Sweden the EBC (SensAbues®) has been used as a screening tool to test those apprehended on suspicion of a drug driving offence but as yet this matrix cannot be collected in a manner that would make it suitable for evidential testing in the British criminal justice system.

3 **INTERNATIONAL APPROACHES TO SETTING CONCENTRATION THRESHOLDS FOR DRUG DRIVING**

A brief review of international practice in terms of drug-driving has shown that countries take different approaches to roadside drug testing both from a legislative and an analytical point of view. The number of drugs targeted differs according to national prevalence, although OF is commonplace as a screening tool. The LLOQ and/or a zero tolerance (LOD) limit seems to be the consensus for illegal drugs, in some cases with the additional requirement of evidence of impairment. A more pragmatic approach is taken with medicinal controlled drugs.

It is becoming increasingly well known that drivers who misuse psychoactive substances may take more than one psychoactive substance together at one time before driving. In many instances this includes the use of alcohol as highlighted in the Technical report ‘Driving under the influence of drugs’.

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The Panel recommends that some discussion is needed with regard to the approach taken when more than one substance is detected in the evidential sample and particularly, whether consideration should be given to substances with a known impairing effect that are present below the level currently set in legislation but may in combination with other psychoactive substances be a risk to driver safety. In some countries a limit has been set for a drug class (e.g., the amphetamines), such that an offence occurs if any combination of the different drugs within the class, when summed, exceed the cut-off.

There is also growing awareness that drugs with similar pharmacological mechanisms of action to those included in the section 5A legislation, but which are not controlled other than through the provisions of the Psychoactive Substances Act 2016, pose similar impairing effects on driving performance. In addition, new evidence is emerging for some drugs controlled under the Misuse of Drugs Act (1971) and these have been included in drug driving legislation elsewhere. Gamma-hydroxybutyrate (GHB) with sedative and anaesthetic effects is a good example. The Panel recommends that the Department for Transport keeps a watchful brief on developments in other drug-driving communities as well as the scientific literature in order to make informed decisions about the addition of further drugs to the section 5A drug-driving legislation.

4 THE CAPABILITY OF UK FORENSIC LABORATORIES TO UNDERTAKE ANALYSES USING EACH MATRIX

It was noted that there are currently (June 29th 2016) seven suppliers with ISO 17025 accreditation for the analysis of drugs under the section 5A offence. All forensic service providers (FSPs) have specific aspects of section 5A for which they are accredited on the UKAS website. All offer the full range of toxicology services although some may use sub-contracting arrangements to achieve this including for the Section 4 offence.

The Panel was informed by the Home Office’s Forensic Marketplace Team that FSPs currently accredited for section 5A blood analysis have some ability to use OF for drug detection purposes and accreditation for OF testing as it is commonly used in workplace drug testing. However, the FSPs are not accredited for the analysis of OF for section 5A purposes. If OF were introduced for evidential testing purposes for the section 5A offence, method development, validation and accreditation would need to take place.

6 https://www.ukas.com/sectors/forensic-science/
The requirement to include multiple drugs with differing cut-off limits in OF should be achievable based on the evidence in the scientific literature and has been accomplished for whole blood. Other agencies such as SAMHSA (The Substance Abuse and Mental Health Services Administration) of the US federal government have also established cut-off for workplace and commercial class drivers. It was noted that FSPs would need to consult on this matter and agree this general principle for this approach.

5 RECOMMENDATIONS OF THE EXPERT PANEL

1. That the Home Office could expand the list of type-approved screening tests to include, in addition to THC and cocaine (which also provides a route to the cocaine metabolite BZE), the amphetamine-type drugs (methamphetamine and MDMA) and ketamine to reflect the growing use of these compounds in driving populations.

2. That whole blood continues to be the most appropriate tool for evidential testing where a per se threshold approach is required such as for medicinal controlled drugs.
   a. Where whole blood is used for evidential tests there should be a specification (minimum standard) for the sample collection kit and the blood collection tube that includes details of the amount of preservative and anticoagulant required.
   b. Assay uncertainty should also be established for the confirmatory test method(s).

3. That OF may be used as an alternative to blood when an LLOQ approach is used.
   a. For evidential testing OF cut-off limits published in the ‘Guide to Type Approval Procedures for Preliminary Drug Testing Devices Used for Transport Law Enforcement in Great Britain’ in 2012 could be used for illicit drugs;
   b. If POCT OF devices are to be used for evidential testing, criteria in terms of sensitivity, specificity and accuracy for OF POCT device(s) should be established by using a type-approval process;
   c. If a commercial device is used to collect OF for evidential purposes, then the recovery of the analytes of interest and the overall reliability of the device should be specified incorporating collection volume, imprecision
data and uncertainty of measurement to provide the drug concentration in neat fluid to satisfy the criminal justice system;

d. A specification (minimum standard) for the sample collection kit and the OF collection tube that includes details of the amount of preservative, stabiliser and buffer should be established;

e. OF samples should be stored in glass tubes, away from fluorescent light and direct sunlight;

f. OF samples should be refrigerated (ideally 3 – 5 °C) as quickly as possible after collection and transported to the laboratory in a controlled temperature to avoid bacterial contamination and degradation of drugs;

g. OF samples should be frozen (ideally at -20 °C), if not available for immediate analysis.

4. That as an adjunct to medical assessment, hair testing is an appropriate matrix for re-licensing decisions, since hair testing provides a much longer window of detection than either blood, urine or OF and would enable the determination of a history of past exposures to illicit or medicinal controlled substances.

5. That a new approach is required where more than one substance is detected in the evidential sample particularly alcohol, where consideration should be given to substances with a known impairing effect that are present below the level currently set in legislation but may in combination with other psychoactive substances be a risk to driver safety.

6. That the Department for Transport should develop a process to monitor changes in recreational drug use patterns and consider new evidence as it becomes available in the scientific literature in order to make informed decisions about the addition of substances to the drug-driving legislation.

7. That the Department for Transport should also keep under surveillance changes in other jurisdictions for potential improvements in their practice of monitoring and deterring drug and drink driving.
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## Glossary of Terms and Abbreviations

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<tr>
<td>Anticoagulant</td>
<td>A product that prevents blood coagulation (clotting)</td>
</tr>
<tr>
<td>Adulteration</td>
<td>In drug testing when a substance or chemical is added to a biological matrix to affect the drug test result</td>
</tr>
<tr>
<td>BAC</td>
<td>Blood alcohol concentration</td>
</tr>
<tr>
<td>BNE</td>
<td>Benzoyl-norecgonine</td>
</tr>
<tr>
<td>BZ</td>
<td>Benzodiazepine</td>
</tr>
<tr>
<td>BZE</td>
<td>Benzoylecgonine</td>
</tr>
<tr>
<td>BZP</td>
<td>Benzylpiperazine</td>
</tr>
<tr>
<td>CAST</td>
<td>Centre for Applied Science and Technology (Home Office, UK Government)</td>
</tr>
<tr>
<td>CE</td>
<td>Cocaethylene – ethyl benzoylecgonine</td>
</tr>
<tr>
<td>COC</td>
<td>Cocaine</td>
</tr>
<tr>
<td>Conjugate</td>
<td>A substance formed by the reversible combination of two or more others; Morphine-3-glucuronide is a conjugate of morphine and glucuronic acid</td>
</tr>
<tr>
<td>Cmax</td>
<td>The maximum concentration of the drug reached in blood after a single dose</td>
</tr>
<tr>
<td>CSEW</td>
<td>Crime Survey for England and Wales (formerly British Crime Survey) is a survey that asks people aged 16 and over living in households in England and Wales about their experiences of crime in the last 12 month (including drug use and driving)</td>
</tr>
<tr>
<td>ABBREVIATION</td>
<td>MEANING</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation / relative standard deviation (standard deviation/mean expressed as a percentage)</td>
</tr>
<tr>
<td>DBS</td>
<td>Dried Blood Spot</td>
</tr>
<tr>
<td>DESI</td>
<td>Desorption electrospray ionization</td>
</tr>
<tr>
<td>DHMA</td>
<td>3,4-dihydroxymethamphetamine is a metabolite of MDMA</td>
</tr>
<tr>
<td>DHMA 3-sulfate</td>
<td>3,4-dihydroxymethamphetamine 3-sulfate is a conjugate of DHMA excreted mainly in urine</td>
</tr>
<tr>
<td>dL</td>
<td>Deci-litre (100mL)</td>
</tr>
<tr>
<td>DMS</td>
<td>Differential mobility spectrometry</td>
</tr>
<tr>
<td>DRUID</td>
<td>Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID) European study</td>
</tr>
<tr>
<td>Ecstasy</td>
<td>MDMA, 3,4-Methylenedioxymethamphetamine is a psychoactive</td>
</tr>
<tr>
<td>EDDP</td>
<td>2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine is the primary metabolite of methadone</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene-diamine-tetra-acetic acid, is an anticoagulant for blood samples</td>
</tr>
<tr>
<td>EME</td>
<td>Ecgonine methyl ester is a major urinary metabolite of cocaine</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>EMCDDA</td>
<td>The European Monitoring Centre for Drugs and Drug Addiction is an agency of the European Union located in Lisbon, Portugal and serving as a centre for drug-related information in Europe</td>
</tr>
<tr>
<td>EMIT</td>
<td>Enzyme multiplied immunoassay technique</td>
</tr>
<tr>
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<tr>
<td>EQAS</td>
<td>External quality assessment schemes provides an overall indication of a laboratory’s performance against external criteria</td>
</tr>
<tr>
<td>FFMT</td>
<td>Forensic Framework Management Team</td>
</tr>
<tr>
<td>First Pass Metabolism / elimination</td>
<td>First-pass metabolism is the fraction of drug lost during the process of absorption and where the concentration of a drug is greatly reduced before it reaches the systemic circulation</td>
</tr>
<tr>
<td>FPIA</td>
<td>Fluorescence polarisation immunoassay</td>
</tr>
<tr>
<td>FSP</td>
<td>Forensic Services Provider</td>
</tr>
<tr>
<td>FSR</td>
<td>Forensic Science Regulator</td>
</tr>
<tr>
<td>FSREU</td>
<td>Forensic Science Regulator’s expanded uncertainty</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography-mass spectrometry</td>
</tr>
<tr>
<td>H</td>
<td>hours</td>
</tr>
<tr>
<td>HMA</td>
<td>4-hydroxy 3-methoxyamphetamine is a metabolite of MDMA</td>
</tr>
<tr>
<td>HMMA</td>
<td>4-hydroxy-3-methoxymethamphetamine is a metabolite of MDMA</td>
</tr>
<tr>
<td>HMMA 4-glucuronide</td>
<td>4-hydroxy-3-methoxymethamphetamine 4-glucuronide is a conjugate of HMMA excreted mainly in urine</td>
</tr>
<tr>
<td>HMMA 4-sulfate</td>
<td>4-hydroxy-3-methoxymethamphetamine 4-sulfate is a conjugate of HMMA excreted mainly in urine</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance (pressure) liquid chromatography</td>
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<td>MEANING</td>
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<tr>
<td>Immunoassay</td>
<td>Biochemical test that measures the presence or concentration of a drug in a solution through the use of an antibody or an antigen</td>
</tr>
<tr>
<td>IEC</td>
<td>International Electrotechnical Commission</td>
</tr>
<tr>
<td>ISO</td>
<td>International Organization for Standardization</td>
</tr>
<tr>
<td>L</td>
<td>Litre</td>
</tr>
<tr>
<td>Lipophilicity</td>
<td>Drug that has an affinity for, or is able to, dissolve in lipids.</td>
</tr>
<tr>
<td>LC</td>
<td>Liquid chromatography</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LLOQ</td>
<td>Lowest Limit of quantification</td>
</tr>
<tr>
<td>LSD</td>
<td>Lysergic Acid Diethylamide</td>
</tr>
<tr>
<td>6-MAM</td>
<td>6-mono acetyl morphine is the primary metabolite of heroin</td>
</tr>
<tr>
<td>MALDI</td>
<td>Matrix-assisted laser desorption/ionization</td>
</tr>
<tr>
<td>MALDI-IMS</td>
<td>MALDI imaging mass spectrometry</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>Matrix-assisted laser desorption/ionization time of flight</td>
</tr>
<tr>
<td>m-CPP</td>
<td>meta-Chlorophenylpiperazine is a psychoactive drug of the phenylpiperazine class</td>
</tr>
<tr>
<td>MDA</td>
<td>3,4-methylenedioxyamphetamine, a metabolite of MDMA</td>
</tr>
<tr>
<td>MDMA</td>
<td>3,4-Methylenedioxyamphetamine is a psychoactive drug commonly known as ecstasy (E)</td>
</tr>
<tr>
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<td>MEANING</td>
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<tr>
<td>MDPV</td>
<td>Methylenedioxypyrovalerone is an illegal stimulant of the cathinone class which acts as a norepinephrine-dopamine reuptake inhibitor (NDRI)</td>
</tr>
<tr>
<td>Mg</td>
<td>Milli-gram</td>
</tr>
<tr>
<td>m-HO-BZE</td>
<td>m-hydroxy-benzoylecgonine is a urinary metabolite of cocaine</td>
</tr>
<tr>
<td>m-HO-COC</td>
<td>m-hydroxy-cocaine is a minor metabolite of cocaine</td>
</tr>
<tr>
<td>mL</td>
<td>Milli-litre</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>MS/MS</td>
<td>Tandem mass spectrometry</td>
</tr>
<tr>
<td>NaF</td>
<td>Sodium fluoride</td>
</tr>
<tr>
<td>Nano</td>
<td>$10^{-9}$</td>
</tr>
<tr>
<td>NCOC</td>
<td>Norcocaine is a minor pharmacologically active metabolite of cocaine</td>
</tr>
<tr>
<td>Ng</td>
<td>Nano-gram</td>
</tr>
<tr>
<td>NHTSA</td>
<td>National Highway Traffic Safety Administration (USA)</td>
</tr>
<tr>
<td>OF</td>
<td>Oral fluid. This is the liquid present in the oral cavity (mouth). Oral fluid is a mixture of saliva and oral mucosal transudate</td>
</tr>
<tr>
<td>11-OH-THC</td>
<td>11-Hydroxy-$\Delta^9$-tetrahydrocannabinol is the main pharmacologically active metabolite of THC which is formed in the body after cannabis is consumed</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio represents the odds that an outcome (for example, being injured in a car incident) will occur given a particular exposure (drug use), compared to the odds of the outcome occurring in the absence of that exposure</td>
</tr>
<tr>
<td>ABBREVIATION</td>
<td>MEANING</td>
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</tr>
<tr>
<td>Pharmacokinetics</td>
<td>Study of the relationship between administered doses of a drug and the observed blood (plasma or serum) or tissue concentrations; what the body does to a drug. The time course of a drug in the body – what the body does to a drug</td>
</tr>
<tr>
<td>Pharmacodynamics</td>
<td>The branch of pharmacology concerned with the effects of drugs and the biochemical and physiological mechanism of their action. The time course of a drug’s effect on a body – what the drug does to a body</td>
</tr>
<tr>
<td>p-HO-BZE</td>
<td>p-hydroxy-benzoylcegonine (a cocaine metabolite)</td>
</tr>
<tr>
<td>p-HO-COC</td>
<td>p-hydroxy-cocaine (a cocaine metabolite)</td>
</tr>
<tr>
<td>POCT</td>
<td>Point of care (collection) testing</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive Predictive Value is the probability that drivers with a positive screening drug test truly have used drugs</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operating characteristic (ROC) curve. A plot of assay sensitivity versus 1-specificity, this illustrates the performance of a binary classifier system as its discrimination threshold is varied</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk or risk ratio (RR) is the ratio of the probability of an event occurring (for example, being injured in a car incident) in an exposed group (drug using) to the probability of the event occurring in a comparison, non-exposed (drug free) group</td>
</tr>
<tr>
<td>RSC</td>
<td>Royal Society of Chemistry</td>
</tr>
<tr>
<td>RTC</td>
<td>Road traffic collision</td>
</tr>
<tr>
<td>RTI</td>
<td>Road traffic incident</td>
</tr>
<tr>
<td>SALDI-TOF-MS</td>
<td>Surface-assisted laser desorption/ionization time of light mass spectrometry</td>
</tr>
<tr>
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<td>MEANING</td>
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<td>--------------</td>
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</tr>
<tr>
<td>SAMHSA</td>
<td>Substance Abuse and Mental Health Services Administration</td>
</tr>
<tr>
<td>SBRI</td>
<td>Small Business Research Initiative</td>
</tr>
<tr>
<td>SERS</td>
<td>Surface-enhanced Raman spectroscopy</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid phase extraction</td>
</tr>
<tr>
<td>Specificity</td>
<td>Measures the proportion of negatives that are correctly identified as such (e.g., the percentage of drug test results correctly identified as not having the drug of interest present – true negatives)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Measures the proportion of positives that are correctly identified as such (e.g., the percentage of drug test results correctly identified as positive – true positives)</td>
</tr>
<tr>
<td>SIL</td>
<td>A stable-isotope-labelled (SIL) analog of the drug is often used as an internal standard. Common labels are deuterium((^{2}\text{H})) and (^{13}\text{C}) – Carbon</td>
</tr>
<tr>
<td>TDx</td>
<td>A proprietary fluorescence polarisation immunoassay (Abbott Laboratories)</td>
</tr>
<tr>
<td>TFMPP</td>
<td>3-Trifluoromethylphenylpiperazine is a recreational drug of the piperazine chemical class.</td>
</tr>
<tr>
<td>THC</td>
<td>(\Delta)-9 tetrahydrocannabinol is the main psychoactive substance found in cannabis</td>
</tr>
<tr>
<td>THC-COOH</td>
<td>11-nor 9-carboxy-delta9-tetrahydrocannabinol, also known as 11-nor-9-Carboxy-THC, 11-nor-9-carboxy-delta-9-THC, 11-COOH-THC, and THC-11-oic acid, is the major metabolite of (\Delta)-9 tetrahydrocannabinol</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethylsilyl ethers are commonly used to derivatise a variety of functional groups prior to GC analysis</td>
</tr>
<tr>
<td>TOF</td>
<td>Time of flight</td>
</tr>
<tr>
<td>ABBREVIATION</td>
<td>MEANING</td>
</tr>
<tr>
<td>--------------</td>
<td>---------</td>
</tr>
<tr>
<td>µ</td>
<td>Micro, denoting a factor of $10^{-6}$</td>
</tr>
<tr>
<td>µg</td>
<td>Micro-gram</td>
</tr>
<tr>
<td>µL</td>
<td>Micro-litre</td>
</tr>
<tr>
<td>UHPLC</td>
<td>Ultra-High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>UKAS</td>
<td>United Kingdom Accreditation Services</td>
</tr>
<tr>
<td>VAMS</td>
<td>Volumetric absorptive micro-sampling</td>
</tr>
<tr>
<td>WADA</td>
<td>World Anti-Doping Agency</td>
</tr>
<tr>
<td>W/V</td>
<td>Weight per volume</td>
</tr>
</tbody>
</table>
1 IDENTIFICATION OF ALTERNATIVE BIOLOGICAL MATRICES FOR DRUG TESTING OF DRIVERS APPREHENDED FOR DRUG DRIVING

1.1 BACKGROUND
The variety of different biological matrices that can be analysed to determine the presence or absence of different psychoactive substances is extensive (Scialli et al., 2015, Sohn, 1982). There are, however, practical limitations with regard to the extent to which biological samples can be used, and the mechanism of collection and supervision of samples are critical to the procedure.

1.2 RELATIONSHIP BETWEEN WHOLE BLOOD AND OF AND URINARY DRUG CONCENTRATIONS
The most commonly used matrices for drug testing are blood, urine, hair and oral fluid (OF). Although the terms “saliva” and “oral fluid” are used somewhat interchangeably in the scientific literature, ORAL FLUID more accurately describes the biological characteristics of this matrix. OF tests are regularly used in drug-treatment settings, the prison service and work-place environments. Urinalysis is routinely used in hospital-based services and ‘doping-control’ in sport, blood in forensic environments and hair analysis for medico-legal cases. Other matrices that are also employed for drug testing include sweat, latent fingerprints and exhaled breath.

Blood is currently the only available matrix for the confirmation (evidential test) of those suspected of committing a section 5A drug-driving offence. However, due to the invasiveness of the procedure and the cost of collection alternative matrices are being investigated. This report seeks to review the feasibility of biological fluids other than whole blood to be used for evidential testing for the compounds contained in the new section 5A offence, which includes 17 compounds (Table 1), for which specific cut-off concentrations approved within the legislation have been set. The terms of reference for this expert panel report are presented in Appendix 1.

The Panel have explored the choice of specimen to use as an alternative to whole blood for evidential drug driving tests. In practice, the presence and or previous use of a drug in the body can be determined in many matrices. Although urine has the advantage of being fairly easy to collect in large volumes and is the biological fluid of choice for laboratory-based drug-testing programmes, the interpretation of urine tests is often

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7 Section 5A was inserted into the Road Traffic Act 1988 by the Crime and Courts Act 2013
8 Urine is also listed for section 4 and is in the section 5A primary legislation
complex with great variability in the excretion of drugs and their metabolites (Vindenes et al., 2012a).

**Table 1**  List of compounds specified in the drug driving (Section 5A) offence

<table>
<thead>
<tr>
<th>Drug or Drug Group</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannabis</td>
<td>Δ-9 tetrahydrocannabinol (THC)</td>
</tr>
<tr>
<td>Amphetamines*</td>
<td>Amphetamine</td>
</tr>
<tr>
<td></td>
<td>Methamphetamine</td>
</tr>
<tr>
<td></td>
<td>Methyleneoxymethylamphetamine (MDMA, Ecstasy)</td>
</tr>
<tr>
<td>Hallucinogens</td>
<td>Ketamine</td>
</tr>
<tr>
<td></td>
<td>Lysergic Acid Diethylamide (LSD)**</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Cocaine</td>
</tr>
<tr>
<td></td>
<td>Benzoylcegonine (BZE)</td>
</tr>
<tr>
<td>Opioids</td>
<td>Morphine</td>
</tr>
<tr>
<td></td>
<td>6-MAM</td>
</tr>
<tr>
<td></td>
<td>Methadone</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>Diazepam, Oxazepam, Temazepam, Clonazepam, Flunitrazepam &amp; Lorazepam</td>
</tr>
</tbody>
</table>

*The spelling of amphetamine is in keeping with the Misuse of Drugs Act 1971 rather than the International Nonproprietary name (INN), amfetamine. ** There is only a single report of the direct effect of LSD on driving (Woody, 1970) but most authors make the logical assumption that LSD use is likely to be detrimental to driving ability based on the distorting effect the drug has on perception (Moskowitz, 1977, Moeller and Kraemer, 2002).

Over the last decade interest has grown in the use of OF for drug screening tests as an alternative to urine. The major advantage of OF over urine is the easy, non-intrusive sampling procedure. Toennes and co-workers (Toennes et al., 2005) compared findings in OF, serum and urine, and concluded that OF was superior to urine in correlating with blood drug concentrations and driving behaviour.
OF has been used to screen for opioid drug use in drug-dependent populations and has been widely used to screen for illicit drug use in drivers (Verstraete, 2005). A clear advantage with OF compared with urine is that it is difficult to differentiate heroin from morphine or codeine ingestion with urine drug testing, whereas 6-MAM and heroin are frequently present in OF following heroin use.

There is a growing need for simple, quick, and reliable methods for the confirmation of drug use in those suspected of driving under the influence of drugs. One major incentive for improving testing is the increasing spectrum of drugs that may be used by drivers. In 2007 drugs and alcohol use were contributing factors in up to 22% of motor vehicle crashes in the USA (NIDA, 2008). The Department for Transport has reported that, in 2015 either drugs (including medicinal drugs) or alcohol, or both, were contributing factors in 11% of fatal accidents in Great Britain (UK Government, 2016). Prevalence is likely be to even greater because additional analyses may not be conducted for drugs if alcohol is above the legal limit.

1.3 METHODOLOGICAL AND ANALYTICAL TECHNIQUES AVAILABLE FOR DRUGS INCLUDED IN THE DRUG-DRIVING LEGISLATION

The standard procedure for large scale laboratory based screening to detect psychoactive drug use typically involves urinalysis and consists of an initial test using an immunoassay (enzyme multiplied immunoassay technique (EMIT), fluorescence polarisation immunoassay (FPIA), radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA) to classify the class of substance present (opiate, amphetamine, etc.). In order to establish the specific compound(s) present, a second confirmation test is recommended following the initial qualitative screening test. The requirement for an initial screening test followed by a confirmatory test is the procedure that is generally used to demonstrate that a drug-driving offence has taken place under the new legislation (Forensic Science Regulator, 2014, Forensic Science Regulator, 2016a, Forensic Science Regulator, 2016b).

It is considered standard practice that the methodology employed for the screening test is qualitatively different from the confirmation test. In the laboratory the confirmation test may involve gas (GC) or liquid (LC) chromatography coupled to mass spectrometry (MS). In a legal setting analytical techniques, particularly chromatography (GC or LC) linked to tandem mass spectrometry (MS-MS) are required (Karsh, 2002).
1.3.1 Screening tests

Immunoassays are the basis for initial screening tests undertaken outside of the laboratory setting. They are at best semi-quantitative and generally only able to identify the class of the drug present. The use of self-contained immunoassay drug testing kits for near-patient/point of collection (POCT)/on-site testing provide rapid results without the need for external laboratory support. Such testing offers advantages in simplicity and ease of performance but this may be offset by potential problems concerning poor sensitivity and specificity. Those interpreting the test result to reach a conclusion with serious consequences such as driving under the influence of drugs need to be sure of the validity of the findings. Drug testing at the roadside can therefore only be carried out with equipment that has been type-approved by the Secretary of State after testing by the Home Office’s Centre for Applied Science and Technology (CAST)\(^9\).

Three classes of drug pose particular problems when using the immunoassay screening test; opioids, amphetamines and benzodiazepines (Nordal et al., 2015)\(^10\). These and other compounds of interest will be discussed in greater detail in the sections that follow.

LSD is also addressed. Although the heyday of use was undoubtedly the 1960s (Wesson, 2011), use still prevails in certain populations today (Sullivan et al., 2015). In the United States of America (USA) the National Survey of Drug Use and Health (2009–2013), recorded a 7.7 % use of LSD among subjects aged 12 – 34 years from a nationally representative sample of non-institutionalized individuals (Palamar et al., 2015); Last year drug use among adults aged 16 to 59 and young adults aged 16 to 24 in the Crime Survey for England and Wales (CSEW) recorded the use of LSD at 0.4 % and 1.2 %, respectively (CSEW, 2015); summarising trends from CSEW, this suggests that LSD is part of the repertoire of illicit drugs in use today.

Early efforts to monitor LSD use were limited because of the difficulty in detecting the drug in biological samples and this in turn hindered the objective assessment of the prevalence of its use (Simpson et al., 1997). Today most laboratories report a very low, negligible level of LSD use but, in practice few routinely screen samples for the drug. It is believed that users would be unlikely to attempt to drive because of the altered state of consciousness associated with the consumption of LSD (Carhart-Harris et al., 2016). Efforts to collect samples in a way that would optimise conditions for its detection may help determine a more accurate picture of the extent of its use.

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\(^9\) Such as the Dräger DrugTest® 5000 (Dräger UK, 2016) and the DrugWipe-2® (Securetec, 2016)

\(^10\) Although clonazepam is not routinely found in immunoassay drug test kits it is part of the Home Office NFFA section 5A panel of drugs to screen.
1.3.2 Novel Approaches for screening Tests/New Developments

1.3.2.1 Surface Enhanced Raman Spectroscopy (SERS)
With the goal of developing roadside drug-screening devices that are not dependent on immunoassay, a Solid Phase Extraction (SPE)-based sampling system has been combined with a portable analyser, the Surface-Enhanced Raman Spectrometer (SERS), using gold- and silver doped sol-gels immobilized in glass capillaries. The detection of several drug classes (amphetamines, cocaine, antidepressants, opioids, and hallucinogens) has been achieved using OF, with a focus on cocaine. A method has been successfully developed that allowed consistent detection of five different drugs in OF at 1 mg/L or less with a 10 minute run-time.

In a focused study, ROC (receiver operating characteristic) curves were used to establish that cocaine could be measured at 50 µg/L more than 90 % of the time. This concentration is 5000 times more sensitive than previously reported SERS measurements of cocaine in OF. The manufacturers future work will incorporate the sol-gel capillaries into a ‘lab-on-a-chip’ as part of a sample kit for possible use by the police in conjunction with a handheld SERS analyser for roadside testing (Farquharson et al., 2011, Inscore et al., 2011).

Small Business Research Initiative (SBRI) studies funded by the Home Office undertaken by Ocean Optics (OceanOptics.com) have also shown that it is possible to measure trace concentrations of cocaine and THC in OF using a combination of a portable Raman spectrometer and low-cost SERS substrates based on gold or silver nanoparticles on paper. The Panel were informed that such measurements are quick and easy to implement however detection down to concentrations required for drug-driving legislation was not currently possible and further developmental work was needed to improve the sensitivity of the SERS substrates (Ocean Optics, 2016).

1.3.3 Confirmatory (Evidential) Tests
Chromatographic methods are generally used to confirm the presence of specific compounds in blood, hair, urine and OF. For legal purposes the gold standard is either GC or LC linked to mass spectrometry (MS), increasingly used in tandem (MS-MS) with stable, labelled, internal standards (Stokvis et al., 2005). This procedure provides definitive quantitative information about the presence of specific compound(s) in the body; the evidential test. It is a requirement of law in England and Wales that confirmatory (evidential) tests are undertaken.

Many laboratories have developed methodological processes for determining several compounds using one analytical technique. For instance, Vindenes et al. (Vindenes et al., 2011) used LC-MS/MS to screen for 32 of the most commonly abused drugs and their
metabolites in 0.5 mL preserved OF, and compared results to urine samples taken at the same time. Patients (n = 45) stabilized on either methadone or buprenorphine provided 164 pairs of OF and urine. Morphine was found more often in urine (n = 66) than in OF (n = 48), whereas the opposite was the case for 6-MAM (n = 20 in urine and n = 48 in OF). Amphetamine was detected less often (n = 45) in urine compared to OF (n = 51) as was methamphetamine (n = 39 vs n = 45) and N-desmethyldiazepam (n = 37 vs n = 51). The other benzodiazepines, cannabis and cocaine were found more frequently in urine samples. It was concluded that, if using a sensitive LC-MS/MS technique, OF would be a good alternative to urine for detection of relatively recent drug use (Allen, 2011).

LC–MS/MS methodologies have been widely applied to the identification and quantification of a range of compounds in biological samples (Maurer, 2005). One of the most interesting developments in the field in recent years has been the direct injection of neat biological samples onto chromatographic systems. Direct injection of urine has been extensively reported using LC–MS/MS for the analysis of different compounds (Dams et al., 2003, Nordgren and Beck, 2004, Nordgren et al., 2005) and similarly plasma (Kollroser and Schober, 2002). The injection of neat urine significantly speeds up the analytical process by removing the need to extract the drug from the matrix (prepare the sample) before introducing to the chromatographic system.

The direct analysis of OF has been less frequently reported. Mortier et al., (Mortier et al., 2002) recorded simultaneous detection of amphetamines, opioids and cocaine by direct analysis of diluted OF samples using LC quadrupole-time-of-flight MS, with electrospray ionization (ESI). However, sample preparation seems to be a rate limiting step and interference introduced by the sample collection devices were problematic and resulted in poor reproducibility of drug detection (Mortier et al., 2001). Sample preparation including protein precipitation, solid-phase extraction, liquid–liquid extraction and Toxi-Tubes extraction still seem to be an important step when seeking to confirm the presence of different drugs in the aqueous medium of OF (Chen et al., 2013).

However this is an emerging field and, more recently, LC-MS/MS has been applied to the simultaneous analysis of OF supernatant for methadone, 2-ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine (EDDP), morphine, 6-MAM, amphetamine and methamphetamine. The OF supernatant was directly injected into the LC-MS/MS system operated under reverse α-phase LC-ESI. Deuterated analogues of the analytes were adopted as the internal standards to compensate for potential matrix effects and LOD were in the ranges of 0.1-1.0 µg/L and 0.25-1.0 µg/L (Liu et al., 2015).
1.3.3.1  The maturity of confirmatory analysis in blood and OF
The majority of the substances included in section 5A of the drug-driving legislation can be easily determined in whole blood in the laboratory. The use of OF as a confirmation test may require more sophisticated equipment but is nevertheless manageable in today’s forensic laboratory. A brief update of recent analytical methods for the drugs included in the section 5A legislation is provided in brief below.

1.3.3.2  Cannabis
Cannabis (THC) can be determined in a variety of different matrices (Thomas, 2015) including dried blood spots (Sharma et al., 2014), OF and hair. OF has been used to measure THC concentrations using different collection tools (Houwing et al., 2012). THC and its metabolites can be determined in urine using LC-MS (Konig et al., 2011, Lee et al., 2013). In 2015, a LC-MS/MS method was successfully applied to the detection of THC in exhaled breath employing a sampling device collecting aerosol particles (Stephanson et al., 2015).

1.3.3.3  Cocaine and BZE
Cocaine and BZE are routinely determined in blood, urine, hair and OF (Lund et al., 2011, Xiong et al., 2013). Specific details can be found in later sections.

1.3.3.4  Ketamine
Detection of ketamine has been achieved by Ultra High Performance (UHP) LC-MS/MS with Dispersive Liquid/Liquid Micro-extraction (DLLME), using an extraction technique that employs microliter amounts of organic solvent, allowing the detection of the studied analytes with limits of detection (LOD) ranging from 0.2 to 2 µg/L in blood (Odoardi et al., 2015). Detection in OF has been achieved using LC-MS (So et al., 2013), in urine (Leung and Baillie, 1989, Ma et al., 2012a) and by LC-electrospray ionization tandem mass spectrometry in hair (Xiang et al., 2011).

1.3.3.5  Lysergic acid diethylamide (LSD)
LSD has been difficult to quantify in biological samples because of the low dose required to achieve a pharmacological effect but, with modern analytical techniques, LSD is easily determined in blood (Pedersen et al., 2013, Martin et al., 2013), urine (Chung et al., 2009, Dolder et al., 2015a, Jang et al., 2015), hair (Jang et al., 2015) and OF (Oiestad et al., 2007). In the latter case the LOD was determined to be 1.8 µg/L and the assay sensitivity and specificity was 90% and 100% respectively.

Analysis of LSD in urine has several issues. Chromatographically the trimethylsilyl (LSD-TMS) derivative is difficult to detect because of the very high retention and deactivation of the sample path and is an important concern (Besserer, 2016, Reuschel et al., 1999).
Scientists have addressed this issue for biological samples with adsorptive stripping voltammetry in Dimethylformamide (DMF)/tetrabutylammonium perchlorate. A linear range of between 1 to 90 µg/L and a LOD of 1.4 µg/L and a LLOQ of 4.3 µg/L were reported (Merli et al., 2014). Monitoring LSD use in the driving population is hampered at the moment as forensic laboratories do not routinely screen for the drug.

1.3.3.6 Opiates and Opioids
A procedure for the simultaneous determination of 12 opioid related compounds in OF using LC-MS/MS has been developed. The importance of sensitive chromatographic methods to separate the various opioids (oxymorphone, hydromorphone and morphine have the same nominal molecular weights) has been noted as critical for correct identification (Tuyay et al., 2012).

Methadone is widely used for the treatment of heroin dependence and for pain management programmes and has been analysed in clinical, forensic and traffic medicine for confirmatory purposes by GC-MS with deuterium-labelled internal standards. The analytical LLOQ for methadone and EDDP by GC-MS were 20 µg/L and 3 µg/L, respectively. In clinical samples from patients (n = 46), the concentrations of methadone in plasma and whole blood were highly correlated (r = 0.92, p < 0.001) and mean (median) plasma/blood distribution ratios were 1.43 (1.41), respectively (Concheiro et al., 2008, Concheiro et al., 2011b).

1.3.3.7 Amphetamines
Amphetamine, methamphetamine and MDMA are easily determined using chromatographic methods in blood (Cook et al., 1990, Kolbrich et al., 2008). For instance, a stereoselective LC-MS/MS method for the simultaneous quantification of MDMA, 3,4-methylenedioxamphetamine, and the key metabolites (DHMA, DHMA 3-sulfate, HMMA, HMMA 4-glucuronide, HMMA 4-sulfate, and 4-hydroxy 3-methoxymphetamine) has been developed on standard reverse α-phase stationary phases (Steuer et al., 2015, Schwaninger et al., 2011). Analysis has also been achieved in urine (Franco de Oliveira and Yonamine, 2016), sweat (Gentili et al., 2016), OF (Mortier et al., 2002, Desrosiers et al., 2013) and hair. Detection of amphetamine using LC-UV and precolumn derivatization was achieved in hair within the 2.0-20.0 ng/mg concentration range, with LOD (limit of detection) from 0.25-0.75 ng/mg (Argente-Garcia et al., 2016).

1.3.3.8 Benzodiazepines
There are numerous analytical procedures for the determination of benzodiazepines with GC-MS a popular method in clinical and forensic toxicology. LC-MS has matured as a way forward, reducing the need for derivatization. Whole blood has been used to detect the
presence of benzodiazepines using UHPLC–MS/MS-system (Sauve et al., 2012). The detection of benzodiazepines in OF is not without difficulty since the OF/blood ratio for most of the drug class is low but has been achieved (Moore et al, 2007, Agilent). The laboratory LOD for some common benzodiazepines are shown in Table 2 (Moffat et al., 2011, Sauve et al., 2012).

Table 2  Analytical cut-offs (laboratory limits of detection-LOD) in blood and OF for common benzodiazepines using chromatographic methods

<table>
<thead>
<tr>
<th>Substance</th>
<th>Blood (µg/L) LOD GC-MS (Moffat et al., 2011)</th>
<th>Blood (µg/L) LOD UHPLC–MS/MS (Sauve et al., 2012)</th>
<th>OF (µg/L) LOD LC-MS/MS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazepam</td>
<td>20</td>
<td>0.1</td>
<td>5</td>
</tr>
<tr>
<td>Nordiazepam</td>
<td>20</td>
<td>0.1</td>
<td>5</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>10</td>
<td>1.0</td>
<td>5</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>50</td>
<td>0.2</td>
<td>5</td>
</tr>
<tr>
<td>Temazepam</td>
<td>50</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Clonazepam</td>
<td></td>
<td>0.3*</td>
<td>5</td>
</tr>
<tr>
<td>Flunitrazepam</td>
<td></td>
<td>0.002*</td>
<td>5</td>
</tr>
</tbody>
</table>

*https://www.redwoodtoxicology.com/resources/cutoffs_methods/screen_confirm_oral

1.4 Cut-off levels for screening and confirmatory (evidential) tests
Analytical cut-off levels tend to reflect the concentrations of drugs that are found in different matrices (Tsanaclis and Wicks, 2007); drug concentrations in hair samples are much lower than those found in urine. Each drug or metabolite will have an analytical cut-off (LOD) according to the type of matrix tested (i.e. blood, urine, OF or hair etc.). For example, the cut-off for cocaine in urine will be in micrograms/L and in hair it is nanograms/mg of hair. When testing hair for the presence of THC the cut-off level using GC-MS may be 0.1 ng/mg whereas in OF using the DrugWipe-2® immunoassay test the cut-off for THC is 10 µg/L.

The lower limit of quantification (LLOQ), the lowest concentration that can be reliably measured in a biological sample, but above the concentration of passive exposure varies depending on the compound being analysed. The LLOQ is also dependent on the sample
volume available for analysis, the physicochemical properties of the compound being analysed and the laboratory equipment available to the analyst.

The LLOQ is important with regard to the detection of positive samples. For total morphine analysis, detection in urine would be possible for 24 – 36 h, at a cut-off concentration of 300 µg/L, but is reduced to less than 12 h at a cut-off concentration of 2000 µg/L, which is used by specialist drug treatment settings to confirm heroin dependence (Cone et al., 1996). Cut-offs also vary according to the type of laboratory equipment employed; cut-off levels for screening tests using immunoassays are often greater than those achieved for chromatographic methods. Using a chromatographic method the cut-off level for the THC-COOH metabolite is 0.002 ng/mg of hair (SoHT, 2003).

1.5 Summary
It remains the case that roadside screening to detect psychoactive drug use can be carried out successfully using an OF immunoassay. The Panel felt that the Home Office could expand the list of type-approved drugs, in addition to THC, cocaine (which also provides a route to the identification of the cocaine metabolite BZE), the amphetamine-type drugs (methamphetamine and MDMA) and ketamine. This would help better target evidential tests and would reflect the growing use of these compounds in UK driving populations (EMCDDA, 2013, CSEW, 2015).

A second test is required following the initial qualitative OF screening test. The confirmatory test will involve a chromatographic procedure such as LC-MS (probably in tandem, MS/MS). It is expected that technical developments will continue to take place in the analytical field and these will more likely be concerned with sample preparation.

The Panel acknowledged that the chromatographic analysis of the drugs included in the section 5A offence was mature and was at a level of sophistication that enabled the measurement of low concentrations of drugs in various different matrices including whole blood and OF.

The relative advantages and disadvantages of the different matrices available will be discussed in turn beginning with blood, which is considered to be the gold standard.
Blood drug concentrations identify very recent drug use and provide quantitative information which may be used for therapeutic assessment, in cases involving poisoning, fatalities, or for medico-legal purposes. Blood serum or plasma is used for the examination of drugs such as paracetamol and salicylate in overdose. In forensic settings blood, or retrieved serum or plasma may be used to establish whether drugs contributed to death. Blood is also the preferred matrix for the interpretation of pharmacologically active drug concentrations and provides a means to estimate total body drug concentration, allowing for drug versus dose correlations to be made for diagnostic purposes.

2.1 Detection time of drugs in blood

Whole blood is currently considered to be the most appropriate choice for setting cut-off concentrations; blood concentrations provide an accurate picture of the amount of drug(s) present in the body at the time of sampling and provides the strongest scientific evidence in relation to driving performance. Whole blood is used for practical purposes because blood is usually haemolysed by the time it reaches the laboratory and hence is not suitable for separating into plasma or serum. Also drug concentration in different blood products may differ. For instance, drug concentrations in whole blood may differ from those determined in plasma.

By way of an example, the analysis to determine blood/plasma ratios for different benzodiazepine (BZ) drugs that have been determined by different researchers is presented in Table 3 for information.

Table 3 Blood; plasma ratio data for different BZE (DRUID, 2012)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Blood/plasma ratio</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazepam</td>
<td>0.55 – 0.70</td>
<td>(Skopp, 2004b)</td>
</tr>
<tr>
<td>Nordiazepam</td>
<td>0.59</td>
<td>(Moffat et al., 2011)</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>0.63 – 0.8</td>
<td>(Jantos et al., 2011)</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>0.9 – 1.0</td>
<td>(Shull et al., 1976)</td>
</tr>
<tr>
<td>Flunitrazepam</td>
<td>0.75</td>
<td>(Skopp, 2004b)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>0.65</td>
<td>(Moffat et al., 2011)</td>
</tr>
</tbody>
</table>
For the purpose of evidential testing, whole blood is preferred because standardisation for comparative purposes across laboratories can easily be achieved.

Blood sampling is currently the most common and most effective way to measure, for evidential purposes, the concentration of a drug in the body of those apprehended for possible drug-driving. However following ingestion, many drugs leave the blood stream fairly quickly, which means that the time between the traffic stop or accident and the blood sampling is important. If delayed, the blood drug concentration may have markedly decreased from the concentration at the time of the driving incident, in some cases falling below the legal cut-off. It is therefore important that blood sampling occurs as quickly as possible after the road traffic incident for a successful prosecution to occur.

2.2 Drug residence in blood

The elimination of a specific drug from the body is determined by the physicochemical characteristics of the drug and this can be calculated by a parameter known as the plasma elimination half-life ($t_{1/2}$). This is the time taken for the concentration of the drug in blood to fall by 50% and it is well established that it takes 5 to 7 half-lives for a drug to fall below easily detectable concentrations (Wolff et al., 1999). For instance, based on a half-life of 2.5 h, if a driver had a blood cocaine concentration of 10 µg/L at the time of the driving incident this concentration would be expected to have fallen to about 5 µg/L after 2.5 h.

Table 4 provides the elimination half-lives in blood and, where available, oral fluid (OF) for the illicit drugs included in Section 5A of the drug-driving legislation. As can be seen, the residence time (half-life, $t_{1/2}$) of illicit drugs in OF tends to be shorter than is observed in blood with the exception of BZE. This is particularly the case for THC whose pharmacokinetics are complicated and are best described by a multi-compartment model. Further detail is provided below.

With regards to specific drugs the following applies for blood:

2.2.1 Cannabis

THC appears in plasma immediately after the first inhalation of a cannabis cigarette and peaks near the end of smoking (Huestis et al., 1992). THC is rapidly metabolised to the active metabolite 11-OH-THC, with concentrations peaking 13 minutes after smoking (Desrosiers et al., 2014). The plasma elimination half-life of THC has been described as multiphasic (Moffat et al., 2011); the distribution phase ($t_{1/2\alpha}$) is relatively short since THC is rapidly assimilated and distributed to adipose tissues. $T_{1/2\alpha}$ for regular users (about 2 h)
is marginally different from recreational users (about 1.5 h) of cannabis (Moffat et al., 2004, Moffat et al., 2011).

Table 4   Elimination half-lives (t½) for illicit drugs included in Section 5A of the drug driving legislation in blood and Oral Fluid (OF)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plasma elimination t½ (range, h)</th>
<th>OF elimination t½ (range, h)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ-9 tetrahydrocannabinol (THC)</td>
<td>21.5* (19 – 103)</td>
<td>1.5 ± 0.6 1.4 ± 0.1 1.6 ± 0.4</td>
<td>(Heuberger et al., 2015, Toennes et al., 2005, Kauert et al., 2007, Scheidweiler et al., 2010, Desrosiers et al., 2014)</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>10 – 11.7 (8 – 17)</td>
<td>7.6 ± 2.1</td>
<td>(Cruickshank and Dyer, 2009, Newton et al., 2005, Schepers et al., 2003, Cook et al., 1990)</td>
</tr>
<tr>
<td>Methylenedioxymethylamphetamine (MDMA)</td>
<td>7.2 ± 1.4 (2.7 – 9.0)</td>
<td>5.6 ± 0.9 (4.6 – 7.4)</td>
<td>(de la Torre et al., 2000, Kolbrich et al., 2008, Navarro et al., 2001, Desrosiers et al., 2013)</td>
</tr>
<tr>
<td>Cocaine</td>
<td>1.5 ± 0.1 (0.7– 4.0)</td>
<td>1.2 ± 0.2 (1.1 – 3.8)</td>
<td>(Jufer et al., 2000, Jufer et al., 2006, Moolchan et al., 2000, Jones, 2008, Scheidweiler et al., 2010)</td>
</tr>
<tr>
<td>Benzoylcegonine</td>
<td>6.4 (6.6 – 7.9)</td>
<td>9.2 (3.4 to 13.8)</td>
<td>(Jones, 2008, Moolchan et al., 2000, Scheidweiler et al., 2010)</td>
</tr>
<tr>
<td>Ketamine</td>
<td>2.5 (1.67 – 3.33)</td>
<td>Not known</td>
<td>(Domino et al., 1984, Malinovsky et al., 1996)</td>
</tr>
<tr>
<td>6-MAM</td>
<td>0.6 (0.1 – 0.4)</td>
<td>Not known</td>
<td>(Cone et al., 1991, Moffat et al., 2004, Moffat et al., 2011)</td>
</tr>
<tr>
<td>Lysergic Acid Diethylamide</td>
<td>3.6 ± 0.9 (2 – 4)</td>
<td>Not known</td>
<td>(Schmid et al., 2015, Dolder et al., 2015b)</td>
</tr>
</tbody>
</table>

*THC has several distribution phases (Huestis et al., 1992). An initial distribution phase (t½α) to highly perfused tissues results in a rapid decrease in plasma concentrations, t½α is about 2h. A second distribution phase (t½β) occurs that leads to an accumulation of THC in poorly perfused tissues, which is characterised by a gradual decrease in plasma THC concentration. t½β reported in the literature ranges from 19 h to 103 h.
Prolonged slow release of THC occurs back into the blood stream and enterohepatic recirculation ensures that THC has a relatively long terminal elimination (t½β) half-life (Pulido et al., 2011). The elimination half-life (t½) of THC after oral consumption or smoking is reported to vary between 19 h and 57 h (Ramaekers et al., 2004, Ohlsson et al., 1980, Wall et al., 1983, Goulle et al., 2008), although sampling subjects for up to 72 h (as in many studies) is considered too short leading to an underestimation of this parameter (Johansson et al., 1988, Huestis, 2007). When deuterium labelled THC was given to habitual users (using ≥1 cigarette/day) and blood samples were collected for 10-15 days, the t½β was estimated to be 4.3 days (Johansson et al., 1989) and more recent work suggests >1.5 days (Desrosiers et al., 2014). For further discussion see the review by Wolff and Johnston (Wolff and Johnston, 2014).

2.2.2 Cocaine and BZE

The plasma elimination t½ of cocaine is dose dependent and ranges from 0.7 h to 1.5 h (Laizure et al., 2003, Jones et al., 2008a). Variation in the plasma elimination t½ has been observed following different routes of administration (Cone, 1995). The acute effects of cocaine are measurable 0.5 to 1 h after use (Perez-Reyes et al., 1994, Cone et al., 1994a, Jenkins et al., 1995, Cone, 1995) and are consistent with a concentration of cocaine in the blood greater than 50 µg/L when using an effective dose (Jenkins et al., 2002, Cone, 1995).

Cocaine is extensively metabolised by plasma cholinesterase to benzoylecgonine (BZE), the primary metabolite. BZE is specific to cocaine and hence the presence of BZE is indicative of cocaine use. The identification of metabolites increases the level of certainty of the toxicological determination. Other significant metabolites include ecgonine methyl ester (EME) and ecgonine, which are pharmacologically inactive (Klingmann et al., 2001). A small amount of cocaine is metabolised by N-demethylation to norcocaine, which has significant pharmacological activity (Askin and Diehl-Jones, 2001). Further minor metabolites of cocaine include p-hydroxycocaine, m-hydroxycocaine, p-hydroxy benzoylecgonine (pOHBE), and m-hydroxy benzoylecgonine (Kolbrich et al., 2006).

2.2.3 Ketamine

The detection of ketamine itself in an intoxicated individual is difficult because of the short-acting properties of the drug; the plasma elimination t½ is about 2 h (Malinovsky et al., 1996, Domino et al., 1984), and may pose difficulties for the laboratory if roadside sampling and transportation to the testing site is delayed. Based on the half-life of ketamine, if a driver had a blood ketamine concentration of 10 µg/L at the time of the incident, the blood concentration would have fallen to 5 µg/L after two hours.
It is pertinent to note that when administered orally (recreational use), ketamine undergoes first-pass metabolism in the liver where it is biotransformed into norketamine (through N-demethylation) (Leung and Baillie, 1989). Norketamine is pharmacologically active and, although less active than the parent drug (Leung and Baillie, 1986), the plasma concentrations of this metabolite are three times greater than ketamine following oral administration (Grant et al., 1981). Hydroxynorketamine is an intermediate metabolite in the biotransformation of norketamine into dehydronorketamine. Ketamine metabolites have longer plasma elimination t½ than the parent compound; 5.3 h for norketamine and 6.9 h for dehydronorketamine (Hijazi et al., 2003).

2.2.4 LSD
After little or no research in humans for 40 years, there is renewed interest in using LSD in clinical psychiatric research and practice (Nichols et al., 2016, Rucker et al., 2016). There have been two recently published studies in the literature of the pharmacokinetics and pharmacodynamics of LSD in healthy volunteers (Dolder et al., 2015b, Schmid et al., 2015). Both studies document that following administration of 200 µg of LSD to healthy subjects there were pronounced alterations in waking consciousness that lasted at least 12 h.

The usual adult oral dose is about 100 µg; onset of effects occurs between 40 and 90 minutes after ingestion, followed by peak effects after 3-5 h. Plasma LSD concentrations up to 8.8 µg/L were observed 2 h after ingestion of 160 µg LSD (Upshall and Wailling, 1972) and concentrations following overdose due to insufflation of LSD were between 6.6 µg/L and 16.0 µg/L (Klock et al., 1974). The predominant effects included visual hallucinations, audio-visual synaesthesia, positively experienced derealisation and depersonalization phenomena; effects clearly contra-indicated for driving. The plasma elimination t½ of LSD is about 3.6 h, ranging from 2 to 4 h (Dolder et al., 2015b).

2.2.5 Opiates and Opioids
Heroin is a lipophilic drug, with a plasma elimination t½ of between 2 and 5 minutes, so that following administration it penetrates the blood-brain barrier efficiently to bring about the euphoric ‘rush’ sought after by users. Heroin is rapidly hydrolysed to 6-monoacetylmorphine (6-MAM) a potent metabolite; 6-MAM has a plasma elimination half-life of 6 – 25 minutes (Moffat et al., 2004) and when detected is often seen as conclusive evidence of heroin consumption. The plasma elimination t½ of heroin is too short for the purposes of routine drug detection and would only be detectable in blood for between 10 and 25 minutes after use. Similarly, the detection of 6-MAM would be best achieved between 30 minutes and three hours after a single dose. The likelihood of detection is increased with regular use of heroin.
Morphine is a metabolic breakdown product of 6-MAM. The plasma elimination $t_{1/2}$ of morphine is between 1.5 – 2 h and for the purposes of laboratory analysis the window of opportunity for the detection of morphine would be up to 10 h after ingestion of a single dose (Table 5). In cancer patients at steady-state, receiving 209 mg morphine/day blood concentrations of morphine were found to average 66 µg/L and not inhibit driving performance (Vainio et al., 1995), whilst a single intramuscular dose 8.75 mg/70 kg resulted in a peak 70 µg/L (Berkowitz et al., 1975).

### 2.2.6 Amphetamines

Amphetamine taken orally is well absorbed and has no major pharmacologically active metabolites. The plasma elimination $t_{1/2}$ of amphetamine is about 10 h (Table 4). The $t_{1/2}$ depends to some extent on urinary pH, with alkaline urine leading to retention in the blood ($t_{1/2} = 18 – 34$ h) and acidic urine promoting renal clearance and resulting in a shorter plasma elimination $t_{1/2}$ of $7 – 14$ h (Anggard et al., 1973). After normal therapeutic dosing the plasma concentration of amphetamine is usually less than 100 µg/L (Baylor, 1993). However, ingestion of ten to fifty times the therapeutic amount is not unusual in addicts; in such cases the plasma concentration may be as high as 3000 µg/L. Steady-state blood concentrations of between 2000 µg/L and 3000 µg/L were observed in a regular user (addict) who ingested about 1 g per day (Wan et al., 1978). Several other drugs are metabolized to amphetamine and methamphetamine notably selegeline. Blood concentrations may help distinguish therapeutic use from misuse. A positive relationship between blood amphetamine concentration and impairment has been described with a ceiling reached between 270 µg/L and 530 µg/L (Gustavsen et al., 2006).

#### 2.2.6.1 Methamphetamine

Methamphetamine is usually self-administered by the smoked route, both the free-base form and the hydrochloride salt being volatile. The plasma elimination $t_{1/2}$ of methamphetamine has been reported to be 11.7 h, range 8 h to 17 h (Cook et al., 1990). Maximal blood concentrations of methamphetamine occurred at 2.7 and 2.5 h after intranasal and smoked doses (Harris et al., 2003). Following oral administration of methamphetamine, peak plasma concentrations are seen after 2.6 - 3.6 h and the mean elimination half-life was reported to be 10.1 h, range 6.4 – 15 h (Table 4). Methamphetamine is metabolised to amphetamine, p-OH-amphetamine and norephedrine (Baselt, 2008). Concentrations of 20 – 50 µg/L are typical for therapeutic use, whereas concentrations in recreational use are reported to be in the range 10 to 2500 µg/L (median 600 µg/L). Peak blood methamphetamine concentrations occur shortly after intravenous use, a few minutes after smoking, much more quickly than after oral dosing (NHTSA, 2016). Peak amphetamine plasma concentrations occur around 10 h after methamphetamine use (Logan, 2001).
2.2.6.2 MDMA

After decreasing numbers of MDMA seizures in recent years, the Substance Abuse and Mental Health Services Administration (SAMHSA) and the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) have reported increasing MDMA use in the United States and Europe again since 2010 (EMCDDA, 2013, SAMHSA, 2016).

MDMA displays non-linear pharmacokinetics possibly due to the saturation or an inhibition of MDMA metabolism (the demethylenation step) (de la Torre et al., 2000, de la Torre et al., 2004); increases in plasma MDMA concentrations tend to exceed those predicted by an increase in dose (Kolbrich et al., 2008). MDMA reaches peak plasma concentrations between 1.5 and 3 h after ingestion (de la Torre et al., 2004, de la Torre et al., 2000) or longer depending on the dose (median Tmax observed by (Kolbrich et al., 2008) was 7.1 h, range 4.7 – 15 h) and may be slowly metabolised. The plasma elimination t½ of MDMA has been reported to be about 7 h (Desrosiers et al., 2013) and gender differences may occur as a consequence of the influence of CYP2D6 and catechol-O-methyltransferase (COMT) genetic polymorphisms (Pardo-Lozano et al., 2012, Aitchison et al., 2012).

The most prevalent compound for documenting MDMA exposure with the longest detection window are MDMA itself or HMMA; however, detection of HMMA requires a hydrolysis step during plasma analysis (Kolbrich et al., 2008).

2.2.7 Benzodiazepines

Benzodiazepines are predominantly consumed by the oral route and tend to be readily absorbed. They are often categorised according to their pharmacokinetic profile into three groups; those with short half-lives (<3 – 4 h), for example triazolam; those with medium half-lives (8 – 24 h) such as oxazepam and; those with long half-lives (>24 h) including diazepam. Further details regarding individual benzodiazepines can be found in the Expert Technical Panel report on Driving Under the Influence of Drugs (Wolff et al., 2013).
### Table 5
Elimination half-lives for medicinal drugs included in Section 5a of the drug driving legislation

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plasma elimination t½ (h)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>10.4</td>
<td>(Krishnan et al., 2008, Schepers, 2003)</td>
</tr>
<tr>
<td>Morphine</td>
<td>1.5 ± 0.15 (1.5 – 2)</td>
<td>(Rentsch et al., 2001)</td>
</tr>
<tr>
<td>Methadone</td>
<td>24 – 36</td>
<td>(Wolff et al., 1997)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>20 – 100</td>
<td>(Moffat et al., 2011)</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>4 – 15</td>
<td>(Moffat et al., 2011)</td>
</tr>
<tr>
<td>Temazepam</td>
<td>8 – 11</td>
<td>(Moffat et al., 2011)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>30 – 40</td>
<td>(Moffat et al., 2011)</td>
</tr>
</tbody>
</table>

*Very little work has been completed on the pharmacokinetics of controlled medicinal drugs in OF. See review by Drummer (Drummer, 2007)

### 2.3 Drug metabolites in blood
Many psychoactive substances leave the circulatory system rapidly (e.g. heroin, cocaine, nicotine) such that detection of the parent drug may not be easily achieved in blood unless sampling occurs soon after dosing. In such instances when blood concentrations of parent drugs rapidly fall below the laboratory limit of detection (LOD) the primary metabolite (e.g. morphine for 6-monocetyl morphine (6-MAM), and benzoylecgonine (BZE) for cocaine) may be measured instead. This approach has been used in England and Wales for heroin and cocaine and is the reason why both 6-MAM and BZE have specific cut-off concentrations included in the section 5A drug-driving legislation.

### 2.4 Collection methods and devices used for blood sample collection
Although whole blood is currently the matrix of choice for evidential sampling in drug-driving offences, blood can be difficult to obtain and may be difficult to handle in the laboratory. Blood sampling is invasive in nature and there is a requirement for trained personnel and hygienic procedures, and compliance to health and safety standards (Wolff and Strang, 1999). The Panel noted and welcomed the expansion of the number of
healthcare personnel that are now permitted to collect blood samples in the custody suite environment.

Quantitative tests to determine the fitness to perform complex tasks such as driving a car or other vehicle (haulage lorry, train, aeroplane etc.) have currently only been described using blood drug concentrations; the legislation as it stands in Great Britain requires the need to establish the concentration of a specified drug in the body at the time of the driving offence and achieves this by determining a presumption that the proportion of drug in the blood at the time of the offence was not less than in the specimen (Road Traffic Offenders Act 1988 section 15(2)(b).

2.4.1 Consideration of blood sampling time
There is often an unavoidable delay between the witnessed impairment or road traffic incident and the time of blood sampling such that concerns have been raised about the difficulty of relating blood concentrations to the driving incident. The Panel endorsed the need to obtain specimens as soon as possible after the road traffic incident, given the relatively rapid decline of some drugs in blood; ideally setting a time period of between 1 and 2 h for a successful prosecution to occur.

There is also the consideration about the length of time that the sample is likely to be stored prior to submission to the laboratory. This issue has been discussed in the section on drug stability where the requirement for an anticoagulant and a chemical stabiliser to preserve the sample is considered alongside storage facilities.

The Department for Transport provided the Panel with information about the handling of samples by Forensic Service Providers (FSPs). It was noted that all blood samples needed to be chilled as soon as possible after sample collection and during transportation to the laboratory. Currently samples can take up to 2 weeks to be transferred from custody suites to the laboratory. Samples are then analysed providing results for the police force within 4 weeks.

2.5 Stability of the drugs in blood
Drugs present in solution in blood may be labile and sensitive to light (amphetamine, MDMA, LSD), heat (cocaine) or pH (GHB, amphetamine) (Peters, 2007). In addition, drugs may be excreted in a conjugated water-soluble form, which often renders detection more difficult. In other cases degradation may be a problem, for instance cocaine is broken down by the esterases in blood to BZE. Specific details of individual drugs are provided below.
2.5.1 Cannabis
THC is a difficult analyte to measure in biological fluids. It degrades when exposed to light (not direct sunlight) especially in solution. As a solid *C. sativa* L. stored in the absence of direct light at -18 °C, 4 °C, and 22 °C was found to be more stable than cannabis stored under nitrogen (Turner et al., 1973). The loss of THC after exposure to light does not lead to an increase in cannabinol metabolites, but air oxidation in the dark does. Cannabis however can be stored successfully in the dark at room temperature before analysis (Fairbairn et al., 1976). Degradation of THC in acidic solutions, below pH 4, as monitored by GC with flame-ionization, has also been observed (Garrett et al., 1978, Garrett and Tsau, 1974).

There are also important considerations for storage and laboratory handling since THC binds to glass in aqueous solutions; the rate and extent of glass binding is dependent on the surface area, pre-treatment of the glass and the concentration of the drug in solution. A total of 20 % and 40 % THC at 100 µg/L and 50 µg/L, respectively, was bound to 50 mL volumetric flasks but this could be minimized by silyl pre-treatment of the glass. Significant loss of THC from whole blood after 2 months, even when stored at room temperature in silanised glass tubes was observed, while samples stored at 4 °C and −10 °C were relatively stable (Johnson et al., 1984). In addition, THC in solution has been reported to diffuse rapidly into plastics, and to be taken up (70 – 97 %) by the rubber closures used for plasma vials (Garrett and Hunt, 1974). Christophersen reported that the concentration of THC in blood stored frozen, for 4 weeks at −20 °C, was relatively unchanged, unless stored in polystyrene containers, where the loss was 60–100% (Christophersen, 1986).

2.5.2 Cocaine and BZE
The choice of biological fluid for the detection of cocaine is important. It is well documented that because of its instability *in vitro* cocaine is infrequently detected in biological samples. To this end, it is necessary to collect blood samples in tubes containing a fluoride preservative at a concentration of 2 % (Rees et al., 2012a). In the absence of such a preservative there is a rapid conversion of cocaine to BZE in the sample after collection. The use of the preservative NaF (NaF) was found to be only partially successful in stabilizing the analyte in the blood between sampling and freezing before analysis in the laboratory (Musshoff and Madea, 2010).

2.5.3 Ketamine
Ketamine was found to be stable in plasma samples stored at −20 °C for up to 3 month (Gross et al., 1999) and its metabolites were stable in plasma samples stored at −20 °C for up to 2 month (Idvall et al., 1979). Rigorous storage conditions for the accurate estimation
of ketamine metabolite concentrations in blood samples are thought necessary including immediate centrifuging at ambient temperature to avoid loss of hydroxynorketamine (the intermediate metabolite in the biotransformation of norketamine into dehydro norketamine), most likely attributable to the permeation of this compound into red blood cells. The plasma samples were transported at 4 °C within 2 days and were stored at −20 °C for 10 weeks without any change in the concentrations of ketamine, norketamine and dehydronorketamine (Hijazi et al., 2001).

2.5.4 LSD
Li and co-workers studied the stability of LSD in samples under various storage conditions (Li et al., 1998). They demonstrated that the drug was lost at temperatures above 25 °C and that in transparent containers under light the stability of LSD was dependent on the distance between the light source and the samples, the wavelength of light, exposure time, and the intensity of light. Similar findings have been made more recently (Klette et al., 2002). In order to detect LSD in biological samples protection from light is essential.

2.5.5 Opiates and Opioids
The stability of morphine, codeine, and 6-MAM in blood were studied using either dipotassium ethylenediamine tetra acetate or sodium oxalate as an anticoagulant, and with or without the addition of NaF (NaF). Blood samples containing the drugs of interest were stored at two different temperatures (4 °C and -20 °C) and analysed after three freeze–thaw cycles. Opiate concentrations were decreased in all conditions, but the most unstable was 6-MAM. It was found that NaF improved the stability of opiates at all conditions studied, whereas the type of anticoagulant did not affect the stability of opiates. It was concluded that blood samples should be stored at -20 °C in glass tubes containing sodium oxalate and NaF for maximum stability (Papoutsis et al., 2014).

2.5.6 Amphetamines
Amphetamine and methamphetamine stability in spiked plasma samples has been studied over three freeze/thaw cycles at -20 °C and thawing and keeping the sample at room temperature for 3 h, to allow for benchtop stability. No instability of either drug was observed under these conditions (Peters et al., 2003).

2.5.6.1 MDMA
In a stability study MDMA was determined to be stable in whole blood using HPLC with fluorescence detection after storage at −20 °C, 4 °C and 20 °C. Whole blood samples stored at 4 °C for periods longer than 5 weeks were not viable due to the presence of a high amount of background fluorescence originating from matrix degradation products.
However, MDMA itself was found to be very stable in biological matrices (Clauwaert et al., 2001).

2.5.7 Benzodiazepines
Although many benzodiazepines are reported to be stable significant degradation can occur if they are not stored at −20 °C (Peters, 2007). Hydrolysis and reduction are suggested to be involved in their degradation. Degradation of nitrobenzodiazepines (flunitrazepam, clonazepam and nitrazepam) occurs very rapidly, whereas other benzodiazepines do not appear to be as unstable (Skopp, 2004b). At 22 °C nitrazepam and clonazepam were stable in sterile fresh blood containing preservative over 28 days, whereas 25 % of flunitrazepam was degraded. At 37 °C all three drugs were substantially lost (29 – 51 %) over 9 h (Robertson and Drummer, 1998). Flunitrazepam was the most unstable compound being rapidly converted to 7-aminoflunitrazepam during storage. Clonazepam and 7-aminoclonazepam concentrations also rapidly disappeared from whole blood. A loss of up to 80 % and 20 % has been observed during 6 month storage at 4°C and 20°C (Skopp, 2004a).

A major influence in the degradation of benzodiazepines are an increased temperature and the absence of NaF. For ambient temperature and at 4 °C, the time interval between sampling and analysis strongly influences the quantitative determination of benzodiazepines (Drummer and Gerostamoulos, 2002, El Mahjoub and Staub, 2000, Kerrigan, 2008).

2.5.8 Other
Blood specimens stored with NaF and potassium oxalate at 1.67 % and 0.2 %, respectively were found to maintain mephedrone stability better (p<0.001) than those stored with the anticoagulant EDTA and those stored without preservatives (p<0.0001). It was strongly recommended that in order to maintain the highest mephedrone stability in blood, collection tubes should contain NaF / oxalate preservatives and samples should be stored at -20 °C (Busardo et al., 2015).

For further information on drug stability in blood and other matrices the Panel note the very comprehensive review carried out by Peters (Peters, 2007).

2.6 Blood Collection Kits
In the UK, the Faculty of Forensic and Legal Medicine has oversight of the blood test kits used for the collection of blood for driving offences and the type of equipment employed (needle and syringes). The Panel noted that in recent times alternative blood collection tubes had been investigated. Good practice suggests that a standard specimen collection
kit should be required meeting strictly defined criteria and that they should comply with PAS377:2012.

Vacutainer®, or similar blood collection tubes have reported advantages over other systems with regard to the ease of collection, safety against needle stick injury and the ability to collect more than one sample from a single injection site. This system is notably compliant with standard practice within the NH and the Panel felt was worth exploring alongside the expansion of healthcare professions permitted to collect blood in the custody suite setting since they would be familiar with this collection system.

In Norway, all blood specimens collected from suspected impaired drivers are received in 4 mL BD Vacutainer™ Plus plastic blood collection tubes (BD Vacutainer Systems, Frankling Lake, NJ, USA) containing 10 mg NaF and 8 mg potassium oxalate, and are stored at 4 °C prior to processing (Kristoffersen et al., 2016). The amount of NaF and potassium oxalate in a 6mL Vacutainer™ tube (NaF, 15.0 mg, potassium oxalate 12.0 mg) should be suitable for evidential sampling in the UK.

The Panel noted that there should also be a specification (minimum standard) for the content of each blood collection kit, which should be as follows:

2.6.1 Specification for standard sample collection kit
- Tamper proof
- In-house evaluation process
- Quality control (testing before use)
- Date of manufacture/batch number

The Panel felt that it would be helpful if the police authority and the FSPs establish written protocols for the collection, storage and dispatch of biological samples to the laboratory, and for the management and oversight of reporting test results. The time of sample collection is mandated and any self-reported information concerning recent consumption of both prescribed and illicit substances should be recorded as standard practice.

It was brought to the attention of the Panel that the Royal Society of Chemistry (RSC) booklet is used as the reference document with regard to the collection of blood for evidential tests in police custody suites in the UK. The latest booklets were published in 2015 but their contents and information were not considered.

The Panel proposed that new guidance should be formulated to take into account the new drug-driving legislation with a defined specification for the blood collection tubes, as well as a process for monitoring the quality of this equipment; random sampling of
collection kits and tubes could take place to confirm preservative and anticoagulant concentrations within agreed published limits (see below). The Panel also felt that a shelf life for blood collection tubes should be published and monitored. It is important that the laboratory acting for the defence are also accredited to the standard required to analyse blood samples submitted under section 5A legislation.

2.6.2 Specification for the content of the blood collection tube
- Sealed tube with a neoprene lined cap - a ‘Teflon® cap’;¹¹
- Glass tubes should be 8 – 10 mL in size;
- Final concentration of NaF should be a minimum 1.5% w/v after the addition of blood;
- Confirmation of NaF content should be maintained through the publication of batch No. /shelf life;
- Final concentration of potassium oxalate or sodium oxalate should contain minimum 0.4% anticoagulant after the addition of blood;
- Confirmation of anticoagulant content should be maintained through the publication of batch no. /shelf life.

2.7 ANALYTICAL UNCERTAINTY AT WHOLE BLOOD LIMITS SPECIFIED IN LAW
The estimation of the analytical uncertainty has become a significant issue in the quality control of forensic drug testing. The measurement of analytical uncertainty in forensic samples and the incorporation of this analysis into the reporting of results has been achieved using several different approaches.

2.7.1 Alcohol
When measuring blood alcohol concentrations a ‘guard band’ approach has been used by Sweden and Denmark, where the lower 99.9 % confidence interval limit for the mean of replicate results must be in excess of the limit (i.e. the measured mean minus three standard deviations). In the UK, this ‘coverage factor of three’ (measured mean minus three standard deviations) is also used, and an allowance of 6 mg/dL (or 6 % of the result, whichever is greater) is made. This is then subtracted from the measured mean alcohol test result following duplicate analysis. The result of the analysis is then reported as being ‘not less than’ this lower figure (result – 6 mg/dL, or 6 %) (King and Lawn, 1999, Gullberg, 2012). An expanded uncertainty using the measured mean minus three standard

¹¹ The use of an inert plastic such as Nalgene decreases the likelihood of chemical interference; inert liners (e.g. polytetrafluoroethylene (PTFE), or Teflon) minimise drug adsorption.
deviations gives a confidence interval of 99.7 %. This approach was used in a recent verification study on blood ethanol measurement (Sklerov and Couper, 2011).

2.7.2 Drugs
The introduction of the Section 5A drug-driving legislation has prompted a fresh look at the use of analytical uncertainty. Not only are drugs present in much lower concentrations in blood than alcohol, the assay used must be flexible enough to respond to the range of drugs that may be present in a single sample. The analysis of whole blood samples for drugs requires an analytical method that can detect several different drug classes with differing physicochemical properties (CAST, 2014).

Despite the advent of direct injection of a neat biological fluid onto a chromatographic system both blood and OF may require additional steps to isolate, extract and pre-concentrate the drug from the sample. Some drugs may even require additional chemistry to be undertaken before it can be analysed; the derivatisation of THC prior to analysis by GC-MS for example.

The Panel sought advice from Centre for Applied Science and Technology with regard to managing the issue of analytical uncertainty for the new drug-driving offence. Uncertainties in the range 10 – 50 % for the analysis of drugs in blood are consistent with the work of Horwitz (Thompson, 2004), which predicts that standard deviations as a function of analyte concentration increase as the concentration of the analyte decreases. Although rather out of date with modern analytical techniques the principle of an exponential increase in uncertainty with reducing concentration still applies. An analytical method usually has to be capable of quantifying multiple drugs simultaneously, and the scope of the assay (the number of drugs the assay can quantify) is often as important as the assay uncertainty for each drug.

For drug-driving and many other regulatory applications there is a need for a measure of uncertainty that defines an interval about the measurement result ($Y$), within which the value of the measurand $Y$ can confidently lie. The measure of uncertainty intended to meet this requirement is known as the ‘expanded uncertainty’ ($U$) and is obtained by multiplying the standard uncertainty ($u_c(y)$ by a coverage factor $k$; commonly written as $Y = y \pm U$. The coverage factor ($k$) is chosen on the basis of the desired level of confidence required for the interval defined by $U = ku_c$.12

The idea is to create an interval around the analytical result such that there is a 99% certainty that the true value is encompassed within it. DRUID deliverable 1.4.2 produced some analytical uncertainty data (Table 6) for a variety of compounds (DRUID, 2011). Other organisations have also published criteria regarding analytical uncertainty. For instance, the World Anti-Doping Agency (WADA, 2010), recommend a maximum acceptable combined standard uncertainty value of 10% for amphetamine-type substances in anti-doping tests.

Table 6 Predicted relative reproducibility (assay uncertainty, %) data for some substances investigated in the DRUID studies, at the cut-off calculated with the Horwitz equation (adapted from DRUID Deliverable 1.4.2).

<table>
<thead>
<tr>
<th>Drug</th>
<th>DRUID cut-off</th>
<th>Assay uncertainty (σH,%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>0.1 g/L</td>
<td>8</td>
</tr>
<tr>
<td>THC</td>
<td>1 µg/L</td>
<td>45</td>
</tr>
<tr>
<td>Morphine</td>
<td>10 µg/L</td>
<td>32</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>50 µg/L</td>
<td>25</td>
</tr>
</tbody>
</table>

2.7.3 Current standard procedures in use to address analytical variation

The Forensic Science Regulator (Forensic Science Regulator, 2014) has issued analytical requirements for each drug for a potential section 5A offence as follows:

- The analysis must be sufficiently specific for each drug so that the results can be relied on as identifying the concentration of the reported drug;
- The reported result must be the mean of at least two analyses of the sample;
- For each drug the analytical method must have an LLOQ at a concentration equal to or lower than half of the legal limit; Shall have an upper LLOQ at a concentration equal to or greater than five times the legal limit;
- For each drug the analytical method shall have an expanded uncertainty to provide a coverage probability of 99.7% (not accounting for replicate measurement) at the legal limit equal to or less than 50%;
- For each drug the analytical method shall have an expanded uncertainty to provide a coverage probability of 99.7% (not accounting for replicate measurement) at the legal limit equal to or less than the Forensic Science Regulator’s expanded uncertainty (FSREU).
The Panel noted an example provided by the Forensic Science Regulator (Forensic Science Regulator, 2014) for amphetamine as follows.

“A laboratory has determined that its analytical method for amphetamine has an expanded uncertainty of measurement (to provide a coverage probability of 99.7%) of 10%. This laboratory obtains an analytical result of 320.5 μg/L of amphetamine in the submitted blood sample. The laboratory deducts 20% (the FSREU) from the analytical result to produce the figure of 256.4 μg/L. The result is reported as “not less than” 256 μg/L.”

The impact of varying the confidence placed in the measurement by using the expanded uncertainty at measured mean minus two standard deviations (k = 2) and measured mean minus three standard deviations (k = 3) is illustrated in Table 7 below.

<table>
<thead>
<tr>
<th>Limit (μg/L)</th>
<th>Measured Concentration (μg/L)</th>
<th>Lower Concentration of coverage range k = 2, μg/L</th>
<th>Lower Concentration of coverage range k = 3, μg/L</th>
<th>Combined Standard AU (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0</td>
<td>1.2</td>
<td>0.8</td>
<td>20</td>
</tr>
<tr>
<td>1</td>
<td>4.0</td>
<td>2.4</td>
<td>1.6</td>
<td>20</td>
</tr>
<tr>
<td>10</td>
<td>15.0</td>
<td>10.5</td>
<td>8.3</td>
<td>15</td>
</tr>
<tr>
<td>10</td>
<td>20.0</td>
<td>14.0</td>
<td>11.0</td>
<td>15</td>
</tr>
<tr>
<td>50</td>
<td>60.0</td>
<td>48.0</td>
<td>42.0</td>
<td>10</td>
</tr>
<tr>
<td>50</td>
<td>70.0</td>
<td>56.0</td>
<td>49.0</td>
<td>10</td>
</tr>
</tbody>
</table>

It is worth noting that the test being applied is whether a measured value is greater than the legal limit. This is a one-sided statistical test and hence the probability needs to be halved, i.e. 99.7% (0.3%) becomes 99.85% (0.15%) if you use k=3 for the confidence interval.
Analytical uncertainty has been published for a number of different analytical methods are some are reported here for the drugs included in the section 5A drug driving legislation.

2.7.4 Cannabis
THC and its THC-OH and THC-COOH metabolites have been analysed in whole blood using ‘fast’ GC/negative-ion chemical ionisation/MS methodology using a simple liquid-liquid extraction in the concentration range 0.5 to 20 µg/L (Thomas, 2015). Although GC-MS is commonly used for the analysis of cannabis LC-MS also permits the determination of this drug (Purschke et al., 2016, Chu and Drummer, 2002). Increasingly, LC-MS/MS methodology is becoming the norm for the determination of cannabis in a variety of different matrices (Rohrich et al., 2010, Jagerdeo et al., 2009, Gronewold and Skopp, 2011) as shown below (Table 8). Uncertainty data on different assays are widely reported in the literature, and show variation, even when using the same technique.

In Switzerland the Federal Roads Office (FEDRO) is responsible for the definition of the legal concentration limits for controlled substances. These limits are 1.5 µg/L for THC with a confidence interval of 30% of the measured value. This analytical uncertainty means, in practice, that an offence is confirmed with a blood concentration of 2.2 µg/L and is the basis (concentration limit) for prosecution (Senna et al., 2010).
Table 8  
Effect of assay uncertainty (AU) for THC in whole blood (unless otherwise stated) reported in the literature in relation to differing decision limits

<table>
<thead>
<tr>
<th>Drug</th>
<th>Decision limit* (µg/L)</th>
<th>Uncertainty (µg/L)</th>
<th>Uncertainty (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC</td>
<td>0.2</td>
<td>0.08</td>
<td>42</td>
<td>(Lund et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.42</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>THC</td>
<td>25</td>
<td>8.48</td>
<td>33.9</td>
<td>(Teixeira et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>60</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>THC</td>
<td>7.5</td>
<td>1.67</td>
<td>22.2</td>
<td>(Coulter et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>7.95</td>
<td>15.9</td>
<td></td>
</tr>
<tr>
<td>THC</td>
<td>1</td>
<td>0.16</td>
<td>15.6</td>
<td>(Schwilke et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.78</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3.6</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>9.72</td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td>THC</td>
<td>1</td>
<td>0.19</td>
<td>19.2</td>
<td>(Chu and Drummer, 2002)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>7.13</td>
<td>28.5</td>
<td></td>
</tr>
<tr>
<td>THC</td>
<td>10</td>
<td>&lt;51</td>
<td></td>
<td>(Gronewold and Skopp, 2011)</td>
</tr>
<tr>
<td>THC</td>
<td>0.5</td>
<td>0.13</td>
<td>26.1</td>
<td>(Jamey et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.35</td>
<td>17.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3.66</td>
<td>18.3</td>
<td></td>
</tr>
<tr>
<td>THC</td>
<td>0.5</td>
<td>0.14</td>
<td>27.6</td>
<td>(Jamey et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.38</td>
<td>18.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4.44</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td>THC</td>
<td>2.5</td>
<td>0.65</td>
<td>25.8</td>
<td>(Schwope et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>7.4</td>
<td>29.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>16.7</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td>THC</td>
<td>2</td>
<td>0.7</td>
<td>34.5</td>
<td>(Del Mar Ramirez Fernandez et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>2.9</td>
<td>0.6</td>
<td>21.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.6</td>
<td>21.3</td>
<td></td>
</tr>
</tbody>
</table>

Analysis carried out in blood using LC-MS/MS unless otherwise stated; Lund et al, used OF; Schwilke et al, 2011 urine using GC-MS; Chu and Drummer, 2002 in blood using GC-MS

*Standard solution against which analytical uncertainty calculated

The Panel noted that at the specific cut-off in the section 5A legislation (2 µg/L) the percent assay uncertainty ranged between 17.7 % and 34.5 % for THC.
2.7.5 Cocaine and BZE

Chromatographic techniques can easily distinguish and separately measure cocaine and its main metabolites; BZE is routinely determined as a biomarker of cocaine use. The LOD for chromatographic analysis of cocaine and BZE using MS can be <1 µg/L however, this will depend on how the instrument is used. Multicomponent assays will generally have a higher LOD than assays targeting specific compounds. LOD ranging from 0.25 µg/L to 15.0 µg/L have been reported for cocaine and BZE using chromatographic analysis. Percent assay uncertainty levels can be seen for cocaine in whole blood (legal limits set at 10 µg/L) and OF below in Table 9.

Table 9 Assay uncertainty (AU) for cocaine reported in the literature in whole blood (unless otherwise stated) in relation to differing decision limits.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Decision limit* (µg/L)</th>
<th>Uncertainty (µg/L)</th>
<th>Uncertainty (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>3</td>
<td>2.16</td>
<td>72</td>
<td>(Lund et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>18.9</td>
<td>5.67</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Cocaine</td>
<td>2</td>
<td>0.3</td>
<td>15.3</td>
<td>(Xiong et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.3</td>
<td>22.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>13.2</td>
<td>52.8</td>
<td></td>
</tr>
<tr>
<td>Cocaine</td>
<td>10</td>
<td>2.1</td>
<td>21</td>
<td>(Bjørk et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>333</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Cocaine</td>
<td>10</td>
<td>0.3</td>
<td>29.5</td>
<td>(Ruiz-Colon et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>5</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>56.5</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>60</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>Cocaine</td>
<td>25</td>
<td>4.5</td>
<td>18</td>
<td>(Rees et al., 2012b)</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>31.5</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>72</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

Analysis carried out using LC with tandem MS in whole blood except for Lund et al 2011 who used OF and Rees et al, 2012b, who employed GC-MS

*Standard solution against which analytical uncertainty calculated
There is also information in the literature with regard to the analytical uncertainty of BZE (Table 10) as shown below. It can be seen that at the specific legislative cut-off (50 µg/L) the percent uncertainty ranges from between 17.3% and 30.3% for BZE, although Bassan et al, used serum rather than whole blood.

### Table 10

Effect of assay uncertainty for benzoylecgonine reported in the literature in relation to differing decision limits.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Decision limit * (µg/L)</th>
<th>Uncertainty (µg/L)</th>
<th>Uncertainty (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BZE</td>
<td>5.8</td>
<td>4.2</td>
<td>72</td>
<td>(Lund et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>36.2</td>
<td>32.6</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>BZE</td>
<td>2</td>
<td>0.3</td>
<td>13.2</td>
<td>(Xiong et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.5</td>
<td>15.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>3.6</td>
<td>14.4</td>
<td></td>
</tr>
<tr>
<td>BZE</td>
<td>10</td>
<td>2.4</td>
<td>24</td>
<td>(Bjørk et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>240</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>BZE</td>
<td>10</td>
<td>1.9</td>
<td>19.3</td>
<td>(Ruiz-Colon et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>8.7</td>
<td>17.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>172</td>
<td>34.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>162</td>
<td>20.3</td>
<td></td>
</tr>
<tr>
<td>BZE</td>
<td>25</td>
<td>6.8</td>
<td>27</td>
<td>(Rees et al., 2012b)</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>59</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>96</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>BZE</td>
<td>50</td>
<td>15</td>
<td>30.3</td>
<td>(Bassan et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>6.8</td>
<td>27</td>
<td>(Lund et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>59.0</td>
<td>39</td>
<td></td>
</tr>
</tbody>
</table>

Analysis carried out using LC with tandem MS in whole blood except for Lund et al 2011 (OF); Rees et al, 2012b, who employed GC and; Bassan et al, 2011 who used serum.*Standard solution against which analytical uncertainty calculated
2.7.6 Ketamine

In laboratories in Taiwan a one-point calibration at the threshold value is the most commonly used quantitative approach for estimating measurement uncertainty in urine for drugs of abuse like ketamine (Ma et al., 2012a). This approach is based on the premise that the standard deviation is often increased with greater drug concentration. Therefore, the slope and standard deviation of the calibration calculated from a non-weighted multiple-point calibration curve was not felt to be the best estimate for the threshold. It has been reported that one-point calibration shows similar performance and better efficiency with respect to time and work-load than multiple-point calibration (Bjørk et al., 2010).

From the literature, the percent uncertainty for ketamine in whole blood is reported between 13% and 16% at the concentration of ketamine in whole blood nearest the legal threshold in the section 5A offence (Table 11).

Table 11 Effect of assay uncertainty (AU) for ketamine reported in the literature, in whole blood unless otherwise stated, in relation to differing decision limits

<table>
<thead>
<tr>
<th>Drug</th>
<th>Decision Limit* (µg/L)</th>
<th>Uncertainty (µg/L)</th>
<th>Uncertainty (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine</td>
<td>100</td>
<td>0.14</td>
<td>35</td>
<td>(Kristoffersen et al., 2016)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>13</td>
<td>(Wille et al., 2013b)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>12.5</td>
<td>&lt;16</td>
<td>(Bjork et al., 2010)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
<td>10</td>
<td>(Ma et al., 2012b)</td>
</tr>
</tbody>
</table>

Analysis carried out using LC-MS or LC-MS/MS in whole blood except for Wille et al 2013b who used OF.
*Standard solution against which analytical uncertainty calculated
2.7.7 LSD
For LSD the percent uncertainty at 1 µg/L (the legal threshold in the section 5A offence) in whole blood is approximately 30 % when analysed using UHPLC-MS and somewhat greater in OF using the same chromatographic equipment (Table 12).

Table 12 Effect of assay uncertainty (AU) for LSD reported in the literature in relation to differing decision limits

<table>
<thead>
<tr>
<th>Drug</th>
<th>Decision* Limit (µg/L)</th>
<th>Uncertainty AU (µg/L)</th>
<th>Uncertainty AU (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSD</td>
<td>0.2</td>
<td>0.15</td>
<td>75</td>
<td>(Lund et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>0.27</td>
<td>30</td>
<td>(Pedersen et al., 2013)</td>
</tr>
</tbody>
</table>

*Lund et al, 2011 used OF

2.7.8 Opiates and opioids
A variety of different methods are available for the detection of opiate drugs such as morphine in blood (Goldberger et al., 1994). Using GC-ion trap-MS/MS, recoveries for morphine, codeine and 6-MAM ranged 50 % to 95 % in blood (Rees et al., 2012b), with within-day and intermediate precisions of ≤14 % and ≤12 %, respectively. Percent uncertainty levels reported in the scientific literature for the opioids morphine, 6-MAM and methadone can be found in Table 13.
Table 13  Assay Uncertainty (AU) data for the detection of opioid drugs included in the section 5A drug-drive legislation, in whole blood unless otherwise stated

<table>
<thead>
<tr>
<th>Drug</th>
<th>Legal threshold (µg/L)</th>
<th>Decision * Limit (µg/L)</th>
<th>Uncertainty (µg/L)</th>
<th>AU (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>80</td>
<td>10</td>
<td>4.5</td>
<td>45</td>
<td>(Bjørk et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>330</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-MAM</td>
<td>5</td>
<td>0.7</td>
<td>0.357</td>
<td>51</td>
<td>(Lund et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>4.1</td>
<td>1.722</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methadone</td>
<td>500</td>
<td>6.3</td>
<td>1.134</td>
<td>18</td>
<td>(Lund et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>5.85</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>13.5</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>165</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.14</td>
<td>34</td>
<td></td>
<td>(Kristoffersen et al, 2016)</td>
</tr>
</tbody>
</table>

Analysis carried out using LC-MS/MS in whole blood except for Lund et al 2011 who used OF.

*Standard solution against which analytical uncertainty calculated

2.7.9 Amphetamines

The determination of amphetamines in urine samples by means of liquid-phase micro extraction was validated, including calculation of measurement uncertainty has been published. The limits of detection were 10 µg/L and 20 µg/L for amphetamine and methamphetamine, respectively. The calibration curves were linear over the concentration range 20 µg/L - 1400 µg/L and a relative analytical uncertainty of 2 % (coverage factor k = 2) was calculated (Franco de Oliveira and Yonamine, 2016).

The percent uncertainty levels published in the literature can be seen for amphetamine-type drugs in whole blood (unless stated otherwise) in Table 14. The concentrations of the amphetamine-type drugs reported in the literature of spiked standard blood samples were very much lower for amphetamine than the section 5 legislation requires (250 µg/L amphetamine). It is expected that the analytical uncertainty for amphetamine in whole blood will be similar to that of methamphetamine at about 30 % or lower as for MDMA 24 %.
Table 14  Effect of assay uncertainty (AU) for amphetamine-type drugs in whole blood (unless otherwise stated) reported in the literature in relation to differing decision limits

<table>
<thead>
<tr>
<th>Drug</th>
<th>Legal Threshold (µg/L)</th>
<th>Decision * Limit (µg/L)</th>
<th>Uncertainty (µg/L)</th>
<th>AU (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>250</td>
<td>0.7</td>
<td>0.567</td>
<td>81</td>
<td>(Lund et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>4.2</td>
<td>3.024</td>
<td></td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Meth-Amphetamine</td>
<td>10</td>
<td>1.3</td>
<td>0.74</td>
<td>57</td>
<td>(Lund et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>7.8</td>
<td>2.34</td>
<td></td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>19.4</td>
<td>6.40</td>
<td></td>
<td>33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>121.2</td>
<td>32.72</td>
<td></td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>MDMA</td>
<td>10</td>
<td>50</td>
<td>22.4</td>
<td>44.7</td>
<td>(Bassan et al., 2011)</td>
</tr>
<tr>
<td>MDMA</td>
<td>10</td>
<td>2.4</td>
<td></td>
<td>24</td>
<td>(Bjørk et al., 2013)</td>
</tr>
<tr>
<td>MDMA</td>
<td>1000</td>
<td>240</td>
<td></td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

Analysis carried out in blood using LC-MS/MS unless otherwise stated; Lund et al, used OF; Bassan et al, 2011 used serum.

*Standard solution against which analytical uncertainty calculated

2.7.10 Benzodiazepines
Most recently Kristoffersen et al (Kristoffersen et al., 2016) determined the analytical uncertainty for twenty drugs and alcohol concentrations in whole blood specifically for driving under the influence cases. Diazepam was used as a model substance (alongside THC and alcohol) to establish a new model for estimating the safety margins. A Bayesian modelling approach was used to determine the parameters in the model, using a dataset...
of 4700 diazepam positive specimens, the safety margins for diazepam were 19.5 % (≤2 µM) and 34% (>2 µM), respectively (Table 15).

Table 15  Effect of assay uncertainty (AU) for the benzodiazepine diazepam reported in the literature in whole blood unless otherwise stated

<table>
<thead>
<tr>
<th>Drug</th>
<th>Legal threshold (µg/L)</th>
<th>Decision Limit* (µg/L)</th>
<th>Uncertainty (µg/L)</th>
<th>AU (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazepam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Lund et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>3.6</td>
<td>1.188</td>
<td></td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Diazepam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Wang et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>0.03</td>
<td></td>
<td>16.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.2</td>
<td></td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>11.7</td>
<td></td>
<td>11.7</td>
<td></td>
</tr>
<tr>
<td>Diazepam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Simonsen et al., 2010)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.6</td>
<td></td>
<td>28.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1</td>
<td></td>
<td>19.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>5.6</td>
<td></td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>33</td>
<td></td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>Diazepam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Bjork et al., 2010)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.7</td>
<td></td>
<td>27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>300</td>
<td></td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Diazepam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Kristoffersen et al, 2016)</td>
</tr>
<tr>
<td></td>
<td>550</td>
<td>60</td>
<td>1.14</td>
<td>34</td>
<td></td>
</tr>
</tbody>
</table>

Analysis carried out in whole blood using LC-MS/MS unless otherwise stated; Lund et al, 2011 and Simonsen et al, 2010 used OF; Wang et al, 2012 used urine.

*Standard solution against which analytical uncertainty calculated

There are many examples of methodology used to determine the presence of different benzodiazepines in the laboratory (Ming and Heathcote, 2011). More recently, efforts have been focussed on the development of LC-MS/MS methods for the simultaneous detection and quantification of benzodiazepines with other drugs of abuse such as amphetamines, opiates and opioids in urine. For instance, Schaefer et al (Schaefer et al., 2013) developed and validated a method using online extraction performed by an ion-
exchange/reversed-phase turbulent flow column, reportedly reducing sample preparation time. They achieved LOD of < 10 µg/L and LLOQ of < 25 µg/L.

Determination of assay uncertainty has been reported for other benzodiazepines listed in section 5A including oxazepam (per se threshold 300 µg/L), and lorazepam (per se threshold 100 µg/L) as can be seen in Table 16. The percent uncertainty for lorazepam at 50 µg/L ranged between 27 % and 49 %.

Table 16   Effect of assay uncertainty (AU) for a range of benzodiazepines reported in the literature in whole blood unless otherwise stated

<table>
<thead>
<tr>
<th>Drug</th>
<th>Legal threshold (µg/L)</th>
<th>Decision Limit* (µg/L)</th>
<th>Uncertainty (µg/L)</th>
<th>AU (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxazepam</td>
<td>300</td>
<td>0.6</td>
<td>0.34</td>
<td>57</td>
<td>Lund et al, 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.6</td>
<td>1.30</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>1.14</td>
<td>34</td>
<td>(Kristoffersen et al, 2016)</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>100</td>
<td>1.6</td>
<td>0.77</td>
<td>48</td>
<td>Lund et al, 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.1</td>
<td>3.33</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Lorazepam</td>
<td>5</td>
<td>3</td>
<td>60</td>
<td></td>
<td>Simonsen et al, 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>13.5</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>1.65</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Flunitrazepam</td>
<td>300</td>
<td>100</td>
<td>1.14</td>
<td>36</td>
<td>(Kristoffersen et al, 2016)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>50</td>
<td>100</td>
<td>1.14</td>
<td>34</td>
<td>(Kristoffersen et al, 2016)</td>
</tr>
</tbody>
</table>

Analyses carried out in blood using LC-MS/MS unless otherwise stated; Lund et al, and Simonsen used OF.

*Standard solution against which analytical uncertainty calculated

The expert panel agreed that in order to ensure confidence in the evidential test result the level of uncertainty of the laboratory method employed should be known and published. A minimum standard for analytical uncertainty following chromatographic
analysis of different drugs in whole blood should be established guided by the Forensic Science Regulator’s (Forensic Science Regulator, 2014) analytical requirements.

Table 17  Analytical uncertainty at the cut-off levels required for the drugs included in the Section 5A offence

<table>
<thead>
<tr>
<th>Drug</th>
<th>% Uncertainty</th>
<th>Drug</th>
<th>% Uncertainty</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC</td>
<td>16 – 30</td>
<td>Amphetamine</td>
<td>&lt;30</td>
</tr>
<tr>
<td>Cocaine</td>
<td>21 – 30</td>
<td>Morphine</td>
<td>33 – 45</td>
</tr>
<tr>
<td>BZE</td>
<td>17 – 31</td>
<td>Diazepam</td>
<td>7 - 12</td>
</tr>
<tr>
<td>LSD</td>
<td>30</td>
<td>Lorazepam</td>
<td>&lt;33</td>
</tr>
<tr>
<td>MDMA</td>
<td>24</td>
<td>Diazepam</td>
<td>7 – 12</td>
</tr>
<tr>
<td>6-MAM</td>
<td>42 (oral fluid)</td>
<td>Oxazepam</td>
<td>&lt;30</td>
</tr>
<tr>
<td>Ketamine</td>
<td>35</td>
<td>Temazepam</td>
<td>&lt;30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clonazepam</td>
<td>&lt;34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flunitrazepam</td>
<td>&lt;36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methadone</td>
<td>33</td>
</tr>
</tbody>
</table>

The Panel noted that for whole blood an analytical uncertainty based on 3 standard deviations ($k = 3$) for a confidence level of 99.7 %, with a normal distribution (Birch, 2003) was preferred by the Home Office. The scientific evidence in relation to analytical uncertainty at the cut-off levels required for the section 5A offence are summarised in Table 17.

Based on this information, the measurement uncertainty is conservatively placed at between 30 % - 45 % in the μg/L range, which is in line with those reported by DRUID, with the exception of diazepam. By way of comparison, in the DRUID studies the measurement uncertainty that was used in Switzerland was reported to be 30 % and in Denmark 50 % before a positive result was reported (DRUID, 2011, Senna et al., 2010).
2.8 SUSCEPTIBILITY OF BLOOD TO CONTAMINATION OR ADULTERATION

The Panel considered the existing procedures for the collection and storage of blood samples for evidential tests and agreed that only authorised collection kits should be used for the collection of blood or any other specimens in the drug driving context. The Expert Panel noted that there were variations in the quality of different blood test kits available for this purpose e.g. in the susceptibility of the containers used to tampering.

Although blood properly collected and packaged is very difficult to contaminate and adulterate, for drug-testing purposes there is a need to have a valid and reliable chain of custody between the individual providing the sample and the authority responsible for sample collection that includes the laboratory undertaking the analysis. In human sport anti-doping testing, blood samples are collected using two Vacutainers sealed separately in secure containers with tamper evident caps.

2.9 BLOOD - SUMMARY

Whole blood is currently the matrix used for evidential testing in those suspected of committing a drug-driving offence. This is based on the sound principle that drug concentrations in blood provide an accurate picture of the amount of drug (s) present in the body at the time of sampling and presents the strongest scientific evidence in relation to driving performance. It remains the gold standard in this regard for thresholds based on risk estimates for driver safety.

However, care needs to be taken when collecting blood to ensure that sample integrity is assured; appropriate use of a preservative and an anticoagulant is mandated. Standardisation of the sampling kit and blood collection tubes are warranted with attention given to temperature and light during storage and transportation, and the timeliness of sample collection in relation to the driving incident. As well, those with responsibility for the arrested driver should be aware of the importance of the timeliness of sample collection in relation to the driving incident.

The Panel recommends that where whole blood is used for evidential tests there should be a specification (minimum standard) for the sample collection kit and the blood collection tube that includes details of the amount of preservative and anticoagulant required. The Panel also recommends moving towards the use of a vacutainer blood sampling device for safer sampling and in keeping with current practice in the NHS.
3 URINE

In clinical and some work-place settings, urine has been a preferred biological fluid for the large scale routine analysis of illicit drugs, pharmaceutical drugs and their metabolites. Urine in these settings offers the advantages of high drug/metabolite concentration coupled with reasonably long detection times; however, interpretation is limited as analytical results solely indicate past exposure and the matrix is highly susceptible to adulteration (Kirsh et al., 2015, Mehta et al., 2015). Whilst this is a big issue in workplace drug-testing those apprehended for drug-driving offences are arguably less likely to be able to adulterate their sample. Needless to say considerations such as the dignity of the donor and ease of sample collection (whether to observe voiding) have led to the consideration of other biological fluids in some settings.

The results of urine sample analysis, can only provide retrospective information about drug use rather than tell us about ‘here and now.’ The period of time that the tester wishes to consider influences the choice of body fluid. In addition with urine there is a time lag between the consumption of a drug and its appearance in this biological fluid. This can make the relationship between urinary drug concentration and behaviour difficult to describe. With the exception of alcohol (ethanol), it is generally accepted that urinary drug concentrations are much less useful as a tool for investigating the behavioural effects of a drug on human performance including driving.

3.1 DRUG DETECTION TIME IN URINE

An important issue for consideration is the interpretation of urine drug test results, since the biological data provided by urine analysis is complex and knowledge of the pharmacokinetics of the consumed substance is necessary. There is wide variability in urine drug concentrations observed in individuals, even in those who have consumed the same drug dosage under the same circumstances because urine represents an ‘end-point’; the excretion of a drug from the body.

3.2 DRUG RESIDENCE AND URINE COLLECTION TIME

Collection time with regards to urinalysis is less of an issue when compared to blood sampling because sampling reflects the excretion patterns of drugs rather than their pharmacological activity. Some very fast acting drugs never materialise in urine because they are entirely broken down in the body and evidence of consumption is only provided by metabolic by-products (e.g. heroin to 6-MAM), whilst other very long acting drugs remain detectable (after cessation of dosing) in urine long after the pharmacological effects of the drug have ceased (e.g. cannabis, methadone and diazepam).
Some of the more commonly detected drugs included in the new legislation were considered by the Panel and are discussed separately below:

### 3.2.1 Cannabis
Following smoking, or oral consumption many cannabis derivatives can be detected; over 20 metabolites have been identified in urine and faeces (Widman et al., 1975b, Widman et al., 1975a). THC and 11-OH-THC (11-hydroxy- Δ⁹- THC), the major psychoactive constituents of cannabis are present in low amounts (30 % and 2 %, respectively (Jenkins et al., 1995, Fraser and Worth, 2004) and passive exposure to marijuana smoke may also produce detectable urinary metabolite concentrations (Huestis et al., 1996), if exposure to smokers takes place in a closed environment over time.

Eighteen acidic metabolites have been identified in urine (Huestis et al., 1996) and most of these metabolites form a conjugate with glucuronic acid, which increases its water solubility. THC-COOH is the primary glucuronide conjugate in urine, while 11-OH-THC is the predominant form in faeces (Huestis and Cone, 1998). It is commonly reported that cannabis can be detected for many days (up to 28 days) when urine is used for screening purposes. This finding is related to the presence of the active metabolite 11-OH-THC, for which a half-life of 120 h has been reported, in frequent users (infrequent users 144 h) of the drug (Moffat et al., 2004). The inactive metabolite THC-COOH, which is also detectable in urine for a considerable period of time (Biecheler et al., 2008) has been detected for up to 3 days (range; 2-7 days) after a cannabis cigarette (Grotenhermen, 2003). THC-COOH is agreed to be a much less specific pharmacodynamic indicator of the impact of cannabis on driving performance than THC.

As the plasma elimination t½ for THC metabolites is longer than the plasma elimination t½ of THC itself, detection in urine can be prolonged. Urinalysis immunoassay drug screening tests that detect combinations of cannabis metabolites may detect the presence of cannabis for several weeks (Grotenhermen, 2003). Prolonged positive immunoassay drug test results can also arise due to enterohepatic recirculation of THC and the presence of non-active conjugated glucuronides such as THC-COOH. Although the inactive metabolites of cannabis will contribute to the road-side screening test, it is unclear whether a combination of metabolites alone would push the screening test conducted at the roadside above the screening threshold.

### 3.2.2 Cocaine and BZE
The excretion patterns of cocaine following different routes of administration are complex. Eight metabolites have been identified in urine including benzoylecgonine (BZE), ecygonine methylester (EME), norcocaine (NCOC), benzoyl-norecgonine (BNE), m-hydroxy-
BZE (m-HO-BZE), p-hydroxy-BZE (p-HO-BZE), m-hydroxy-COC (m-HO-COC), and p-hydroxy-COC (p-HO-COC) (Cone et al., 2003). Only about 1% to 5% of cocaine is excreted unchanged into urine, where it can be detected for 3 – 6 h after use (Preston et al., 2002, Preston et al., 1999).

Urine specimens collected for a minimum of three days after drug administration, screened by immunoassay (EMIT and TDX, LLOQ 300 µg/L), and analysed by GC-MS (n = 6) showed that the mean C_max concentrations were in the following order; BZE > EME > COC > BNE approximately p-HO-BZE > m-HO-BZE > m-HO-COC > NCOC > p-HO-COC (Cone et al., 2003). Urinary excretion patterns of cocaine metabolites using BZE equivalents from 18 chronic cocaine users, housed for up to 14 days on a closed research unit, found that 63% of participants tested positive longer than the expected 48 h window of detection after admission to the unit. Mean time to the last positive test after self-reported use of cocaine, was approximately 81 ± 34 h (range 34 – 162 h) (Preston et al., 2002). Standard practice when using urine to test for the presence of cocaine is that the major metabolite BZE is used to confirm the use of cocaine. Benzoylecgonine ethyl ester (cocaethylene, BE-Et) is only identified following co-administration of cocaine and alcohol (Jatlow, 1993).

3.2.3 Ketamine
The presence of ketamine and its metabolites can be detected in urine using GC-MS (Parkin et al., 2008, Turfus et al., 2009) as well as other analytical methods. The window of detection varies depending on the dose, individual variability and the methodology used but, is usually within 48 to 72 h if the major metabolite (nor-ketamine) is included in the analysis.

3.2.4 LSD
Only small amounts of LSD are eliminated unchanged in urine. Using radioimmunoassay urinary LSD concentrations ranging from 1.5 to 55 µg/L were measured within a 24 h period following a 300 µg dose of LSD (Taunton-Rigby et al., 1973). Urine samples may remain positive for the drug for up to 120 h (5 days) after ingestion (McCarron et al., 1990).

3.2.5 Opiates and Opioids
Using urinalysis to interpret opioid use is complex. Heroin (diacetyl morphine), is broken down rapidly and is only present in urine in the form of metabolites (e.g. 6-MAM, morphine, nor-morphine, etc.). The presence of morphine alone or its conjugate may be indicative of clinically administered diacetylmorphine or medicinal morphine (within the
previous 48 h) and therefore of relevance to the drug-driving legislation, but also may indicate illicit heroin use.

The detection of 6-MAM provides a more specific indication of heroin consumption than morphine, which can be prescribed as a drug in its own right, but is usually only present in urine for 12 to 18 h after heroin use. The presence of both morphine and codeine together in urine is thought to be consistent with the ingestion of codeine; though only when the codeine concentration is much greater than that of morphine (Hawks and Chiang, 1986). Although the presence of codeine in urine may indicate illicit drug use, its presence in cough-medicines or analgesic preparations makes such an observation difficult to confirm. Efforts have been made to detect minor components of illicit diamorphine as markers of illicit, as opposed to pharmaceutical heroin use e.g. acetyl codeine and meconin but, these markers are not routinely assayed and further work would be needed to confirm their suitability for the drug-driving context (Morley et al., 2007, Paterson et al., 2005).

The detection of methadone, a long-acting opioid, is easily achieved in urine. However, it is standard practice to test for the presence of 2-ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine (EDDP), its non-active primary metabolite as a means of confirming ingestion (Wolff et al., 1999). More recently the direct injection of the neat sample (‘dilute and shoot’) onto the chromatographic system (LC-MS/MS) has been described (VP., 2012).

3.2.6 Amphetamines
Positive urine test results for either amphetamine or methamphetamine generally indicate use over the previous 1-4 days and urine test results would be difficult to reconcile with a driving incident. Over a 24 h period 60 % of a dose of $^{14}$C amphetamine was excreted in urine, of which 20 % was as unchanged drug and 1 % as the primary amine (Caldwell, 1976). The rate of urinary excretion is heavily influenced by urinary pH. In urine of pH 6 – 8, about 22 % of an amphetamine dose was excreted as unchanged drug, 15 % as $p$-hydroxy amphetamine and 1 % as $p$-hydroxyamphetamine (Shimosato et al., 1986).

3.2.6.1 Methamphetamine
Methamphetamine is readily absorbed from the gastrointestinal tract after oral consumption and is almost entirely (90 %) eliminated in urine over 2-3 days following a single dose. Between 30 – 54 % of an oral dose of methamphetamine is excreted in urine as unchanged methamphetamine and 10 – 23 % as unchanged amphetamine, whereas following an intravenous dose, 45 % is excreted as unchanged parent drug and 7 % amphetamine (Baselt, 2008).
Amphetamine is a major active metabolite of methamphetamine and wide ranging concentrations of urinary methamphetamine from 24,000 µg/L to 33,300 µg/L (24 to 33.3 mg/L) and amphetamine 1000 µg/L to 90,000 µg/L (1 to 90 mg/L), respectively, were observed in drug users of the drug (Lebish et al., 1970).

### 3.2.6.2 MDMA

In urine samples collected from recreational MDMA users following a controlled single dose of MDMA, DHMA 3-sulfate, HMMA 4-sulfate, and HMMA 4-glucuronide were detected as major metabolites next to unchanged MDMA. All other metabolites were detectable in urine, however in insignificant amounts (de la Torre et al., 2004).

### 3.2.7 Benzodiazepines

The benzodiazepines are a large, varied group of compounds that are generally extensively broken down and excreted in the urine as pharmacologically inactive conjugated metabolites. Some metabolites however do possess some pharmacological activity of their own such as nor-diazepam (N-desmethyldiazepam), thus prolonging the effects of the parent drug diazepam (Laloup et al., 2007). Interpretation of urine drug test results may also be complicated since oxazepam is a common urinary metabolite of several benzodiazepines such as diazepam and temazepam thus it may be difficult to determine the parent drug with urine testing. The duration of detectability in urine is varied because the plasma elimination t½ of different benzodiazepines differs substantially (Table 5). When benzodiazepines are misused they are often consumed much in excess of recommended dosing, which may impact on the detection window.

### 3.3 COLLECTION METHODS AND DEVICES USED FOR URINE SAMPLE COLLECTION

The use of self-contained drug testing kits for near-patient/point of contact/collection (POCT), on-site testing is commonplace as a means of drug screening using urine. There are a significant number of on-site tests/brands available today, marketed to test for a variety of different substances; commonly opiates, amphetamines, cocaine metabolite (BZE), benzodiazepines, methadone, and cannabis.

POCT devices provide rapid results without external laboratory support and usually offers advantages in simplicity and ease of performance but this may be offset by potential problems concerning their subjective nature and in some instances the lack of a positive control (Armbruster and Krolak, 1992, George and Braithwaite, 1995b). Those interpreting the test result would need to be sure of the validity of the findings, which may also be difficult to confirm. POCT test kits are generally not used or thought to be suitable for evidential purposes, since in the main POCT devices test for a class of drugs rather than individual substances. POCT tests for urine also tend to employ high cut-offs
that have been agreed for clinical or workplace testing and thus are not suitable for the drug-driving environment.

Currently, where it is necessary to reach a conclusion with serious consequences such as a drug-driving offence, standard practice requires that confirmatory tests are used. In such cases, GC or LC linked to MS is employed. In addition confirmation using chromatography coupled MS techniques can provide definitive quantitative information about the presence of specific drug compound(s) and is suitable for legal requirements.

The results of initial POCT screening tests only usually provides a guide to the type of substance present, and these test results should always be confirmed using a different technique (Karch and Drummer, 2001). For instance, first-generation urine immunoassay screening tests for amphetamine often cross-react with compounds such as ephedrine, found in cough medication to give a false positive result. However, newer immunoassays have reduced this problem. The cross reactivity with its analogues has improved with advancing technology. By way of an example the cross-reactivity of the Immunalysis POCT screening device for amphetamine is shown below (Table 18)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration µg/L</th>
<th>d-Amphetamine equivalent µg/L</th>
<th>(%) Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Amphetamine</td>
<td>25</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>d-Amphetamine</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>d-Amphetamine</td>
<td>75</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>l-Amphetamine</td>
<td>250</td>
<td>24.2</td>
<td>9.7</td>
</tr>
<tr>
<td>d-Methamphetamine</td>
<td>1000</td>
<td>&lt;1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>l-Methamphetamine</td>
<td>5000</td>
<td>&lt;1</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>dl-MDA</td>
<td>200</td>
<td>357</td>
<td>178.5</td>
</tr>
<tr>
<td>(+) Pseudoephedrine</td>
<td>1000</td>
<td>36.8</td>
<td>3.7</td>
</tr>
<tr>
<td>(+) Pseudoephedrine</td>
<td>5000</td>
<td>73.4</td>
<td>1.47</td>
</tr>
<tr>
<td>(-) Pseudoephedrine</td>
<td>500</td>
<td>2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Phenylpropanolamine</td>
<td>5000</td>
<td>10.9</td>
<td>0.22</td>
</tr>
<tr>
<td>(-) Ephedrine</td>
<td>10000</td>
<td>42.4</td>
<td>0.424</td>
</tr>
<tr>
<td>(-) Ephedrine</td>
<td>5000</td>
<td>60.7</td>
<td>1.21</td>
</tr>
</tbody>
</table>

No cross-reactivity was observed for the following drugs (not detected by the immunoassay); dl-methamphetamine (1000 µg/L); dl-MDMA (500 µg/L); dl-MDEA (1000 µg/L); (+) ephedrine (10,000 µg/L); fenfluramine (1000 µg/L); and diphenhydramine (10,000 µg/L).
Newer screening tests such as the EMIT DAU monoclonal immunoassay have fewer problems with cross-reactivity.

3.4 Stability of Drugs in Urine
The stability of methadone, amphetamine, and BZE to different temperatures (60 – 100 °C) at pH 5.1 and 7.6 was investigated (Wolff et al., 1990). Heat-treating urine at 60 °C for 1.5 h or 70 °C for 1 h did not significantly affect the measured concentrations of these drugs. Benzoylecgonine and amphetamine were most susceptible to the different forms of heat treatment. It is worth noting that Galloway and Bellet (Galloway and Bellet, 1999), reported that the GC injector-port temperature (>180 °C) induces thermal conversion of methadone to EDDP as an artefact. They recommended that alternative chromatographic methods (e.g. capillary electrophoresis, LC, or LC-MS) should be considered. Whilst Skopp and colleagues (Skopp et al., 2002), confirmed the importance of the need to protect clinical and forensic urine samples to be analysed for LSD from light and to transport them rapidly to the laboratory.

3.5 Susceptibility of Urine to Contamination or Adulteration
Sample integrity has frequently been an issue with urinalysis, particularly substitution or adulteration of specimens. Collection cups with temperature indicator strips are available for immediate monitoring of specimen temperature. For workplace, pre-employment testing, medico-legal work and sport testing, chain-of-custody procedures, tamper-free collection vessels, and documentation to accompany each sample are required (Wolff et al., 1999). Artificial dilution can be a problem both before (by using diuretic agents widely available on the internet), or after voiding (by adding water) and has led many laboratories to establish criteria for “normally concentrated” or “dilute” urine specimens. As it is possible to drink large volumes of water and lower urine drug concentrations below the positive cut-off, thresholds for tests of urine creatinine and specific gravity have also been recommended (George and Braithwaite, 1995a) and are widely used in workplace testing laboratories.

Measurement of temperature, pH and nitrite level also reduce the probability of adulteration (Wolff et al., 1999). On-site rapid test kits, which require small quantities of biological fluid, are not usually able to benefit from such safeguards. There are also products such as the Instant-View™ Urine ID-Adulteration test device (Instant-View®, 2016) to determine whether urine specimens have been diluted and Urine Specimen Validity (Adulteration) Test (Craig Medical Inc., 2016) for assessing the integrity of urine samples available on the market. Reagents for screening of adulterants using automated
analytical instruments in the laboratory are also available (Axiom Diagnostics Inc., 2016).

Urine can be easily contaminated to increase the probability of false negative results (Dasgupta, 2007) with chemicals (bleach, vinegar, liquid soap) or by dilution. Monitoring the creatinine level or specific gravity of a freshly voided urine sample can help to identify diluted samples (Fraser and Zamecnik, 2003).

3.6 Urine - Summary

Urine reflects drug use over the previous few days (longer with methadone, diazepam and possibly THC) and in this sense is not helpful in the drug-driving context if a relationship between drug impairment and time when consumption last occurred is to be determined. Of importance in determining if urine would make a suitable alternative to whole blood is the time-lag between the consumption of a drug and its appearance in urine. The time-lag may be affected by a myriad of factors such as gender, age, weight and disease state etc., which makes establishing the relationship between urinary drug concentrations and driving behaviour extremely difficult.

Urine drug test results provide information about the manner in which a drug (and its metabolites) is eliminated from the body rather than an indication of drug concentration/activity in the body. Despite the advantage of having a matrix that requires little laboratory preparation and that can be collected in large volumes, urinalysis would present difficulties as a confirmatory test for a prescribed limit drug driving offence.

Although the relationship between blood concentrations and urine concentrations have been researched over many years the general consensus is that urine cannot be used to determine previous pharmacological drug activity in the body. Another major limitation is with regard to the inconvenience of sample collection and lack of integrity (Allen, 2011, Gourlay et al., 2010), which could be a problem in the custody suite unless sample collection was observed. Finally, meaningful results related to a driving offence could not be achieved, even with urinary quantitation (LLOQ approach), due to the variability in individual drug elimination patterns.

The Panel concluded that urinary drug concentrations are not useful as an indicator of the effects of a drug on immediate driver safety, are too wide ranging and therefore unhelpful as a potential alternative to whole blood for confirmatory testing. Although when a zero tolerance approach is employed and a laboratory LLOQ analysis is utilised as the cut-off, then a urine test can be used to support an impairment test result, as is the case for section 4 legislation.
For section 4 the detection of drug(s) in urine is satisfactory to prove their presence when impaired driving had been witnessed (leaving it to the court to decide whether the drug use was the cause of the impairment witnessed). However in a section 5A where there is no (or insufficient) impairment evidence urine cannot be used as there is no other element of evidence that points to the effects of the drugs being present at the time of driving. Urine remains an essential part of the present Section 4 legislation. Details of the drugs included in the section 4 legislation can be found in Section 11.2 of this report in Table 42.
4 **ORAL FLUID (OF)**

Oral Fluid (of which, saliva is a key constituent) has gained popularity as a matrix for drug screening because of the relative ease of sample collection, reduced susceptibility to adulteration (Allen et al., 2005), and because it is a reflection of free drug in the body. Its analysis is becoming more widespread, especially in the monitoring of illicit substances including the opioids (Tuyay et al., 2012, Vanstechelman et al., 2012). The non-invasive nature of OF collection offers an advantage over both blood and urine testing and evidence from a general population survey suggests that OF testing may have lower refusal rates than either hair or urine testing (Fendrich et al., 2004a, Fendrich et al., 2004b, Vindenes et al., 2011).

In the context of drug-driving the use of OF as a drug screening tool offers the ability to test an individual rapidly at the time of a traffic incident. With no requirement for toilet facilities, or same sex collectors, it has become popular in institutions such as prisons. However, POCT (point of contact/collection/on-site testing) devices using OF are only able to offer non-quantitative results rendering them unsuitable as the means by which to provide confirmatory tests, unless specific facilities are available or some part of the sample can be retained separately for laboratory analysis (Boorman and Owens, 2009).

4.1 **DETECTION TIME OF DRUGS IN OF**

Variability in the OF drug concentration and the window of drug detection in OF may depend upon the degree of ionization and physiochemical factors such as pH (Mucklow et al., 1978, De Zeeuw et al., 1980) to a much greater degree than observed with blood. Relative drug lipophilicity is also important since passive diffusion is the major transport mechanism for drugs into OF and explains why cocaine, methadone, codeine and THC predominate over their respective metabolites BZE, EDDP, codeine-6-glucuronide and THC-COOH in OF; the opposite of the picture observed in urine and blood (Kidwell et al., 1998).

With regard to amphetamine-type drugs detection may be variable in OF since the concentrations of these drugs are considerably influenced by salivary pH; an increase in pH (during salivation) results in a reduction of the concentration of amphetamine in OF (Navarro et al., 2001).

Cocaine in particular has been systematically determined in OF and the following table provides examples of chromatographic methods for its detection. Notably the LLOQ ranges between 0.5 µg/L and 10 µg/L and is well within the range required in the section 5A legislation (Table 19).
Table 19  Laboratory LOD reported in the literature for cocaine and benzoylcegonine for confirmatory test purposes.

<table>
<thead>
<tr>
<th>Authors</th>
<th>OF, Sample Volume, μL</th>
<th>LOD μg/L</th>
<th>LLOQ μg/L</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Dams et al., 2007)</td>
<td>200</td>
<td>0.25-5.0</td>
<td>1.0-10.0</td>
<td>LC-MS</td>
</tr>
<tr>
<td>(Schramm et al., 1993)</td>
<td>1000</td>
<td>0.5-10.0</td>
<td>0.5-10.0</td>
<td>GC-MS</td>
</tr>
<tr>
<td>(Scheidweiler et al., 2010)</td>
<td>1000</td>
<td>2.5</td>
<td>2.5</td>
<td>GC-MS</td>
</tr>
<tr>
<td>(Jenkins et al., 1995)</td>
<td>500</td>
<td>1</td>
<td>3.1-6.2</td>
<td>GC-MS</td>
</tr>
<tr>
<td>(Cone et al., 1997)</td>
<td>500</td>
<td>1</td>
<td>1.1</td>
<td>GC-MS</td>
</tr>
<tr>
<td>(Jufer et al., 2006)</td>
<td>500</td>
<td>1</td>
<td>1.25</td>
<td>GC-MS</td>
</tr>
<tr>
<td>(Cone et al., 1994a)</td>
<td>500</td>
<td>N/A</td>
<td>1.6-3.1</td>
<td>GC-MS</td>
</tr>
<tr>
<td>(Kato et al., 1993)</td>
<td>1000</td>
<td>N/A</td>
<td>12.5</td>
<td>GC-MS</td>
</tr>
<tr>
<td>(Vindenes et al., 2012a, Vindenes et al., 2011)</td>
<td>N/A</td>
<td>1-10</td>
<td>1-10</td>
<td>LC-MS</td>
</tr>
<tr>
<td>(Hall et al., 2015)</td>
<td>200</td>
<td>15</td>
<td>15</td>
<td>GC-MS</td>
</tr>
<tr>
<td>(Toennes et al., 2005)</td>
<td>500</td>
<td>8</td>
<td>10</td>
<td>GC-MS</td>
</tr>
<tr>
<td>(Mortier et al., 2002)</td>
<td>200</td>
<td>2</td>
<td>2</td>
<td>LC-MS</td>
</tr>
<tr>
<td>(Clauwaert et al., 2004)</td>
<td>100</td>
<td>1</td>
<td>10</td>
<td>LC-MS</td>
</tr>
<tr>
<td>(Lund et al., 2011)</td>
<td>500</td>
<td>0.3-0.5</td>
<td>0.6-6.0</td>
<td>LC-MS</td>
</tr>
<tr>
<td>(Badawi et al., 2009)</td>
<td>200mg</td>
<td>N/A</td>
<td>0.5μg/kg</td>
<td>LC-MS</td>
</tr>
<tr>
<td>(Langel et al., 2008)</td>
<td>1000</td>
<td>&lt;10</td>
<td>10</td>
<td>GC-MS</td>
</tr>
<tr>
<td>(Concheiro et al., 2008)</td>
<td>500</td>
<td>0.5</td>
<td></td>
<td>LC-MS</td>
</tr>
</tbody>
</table>

4.2 Drug residence in OF

A number of factors affect the residence of drugs in OF and therefore the drug concentration in OF. The pathophysiology of the oral cavity may be important, particularly dry mouth syndrome. Xerostomia is the subjective feeling of dry mouth and the range of the general population reporting xerostomia has been cited in the literature to be between 5% and 46% (Hopcraft and Tan, 2010). Xerostomia is often related to salivary gland dysfunction, a disturbance in the amount or quality of OF being produced; typically manifesting as salivary gland hypofunction (reduced volume of saliva secretion) or a change in salivary composition. Hyposalivation may be diagnosed when salivary flow rates...
are under 0.1 mL/min at rest or 0.7 mL/min under stimulation (Lopez-Pintor et al., 2016, Quock, 2016) and may alter the residence of drugs in OF.

Several circumstances are capable of inducing salivary gland hypofunction, medical conditions including rheumatoid arthritis, diabetes mellitus, hyperthyroidism, hypothyroidism, alcohol abuse (Quock, 2016) and HIV (Aps and Martens, 2005). Salivary flow rate may be reduced by up to 50 % in those diagnosed with alcoholic liver cirrhosis (Aps and Martens, 2005).

Importantly smoking cannabis is associated with a dry mouth (Versteeg et al., 2008, Darling, 2003, Darling and Arendorf, 1992). Darling and Arendorf (Darling and Arendorf, 1992, Darling and Arendorf, 1993) showed that the prevalence of a dry mouth after smoking cannabis, was significantly greater when compared with non-cannabis cigarette-smoking controls (69.6 % and 18.6 %, respectively, P < 0.001).

Exogenous factors such as other drug use may be significant, since both prescribed medication and illicit drug use have been reported to stimulate e.g. ketamine (Yew, 2015) or inhibit e.g. cocaine (Fratto and Manzon, 2014) and morphine (Glare et al., 2006) salivation. Those suffering from a reduced salivary flow or dry mouth syndrome may struggle to provide sufficient OF for confirmatory drug tests.

**4.3 Consideration of OF sampling time**

The detection times for drugs in OF are close to those found in blood and are considerably shorter than those found in urine; ranging from 5 to 48 h for OF compared with 136 – 196 h for urine (Verstraete, 2005). From this viewpoint drug testing using OF may be valuable if the analytical findings can be related to the drug’s equivalent concentration in blood (Verstraete, 2005, Toennes et al., 2005, Liu et al., 2015). For this reason it has been postulated that drug detection in OF if quantified, may reflect the drug’s influence on the specimen donor at the time the biological sample was collected. However, researchers have reported large variations in OF: blood ratios for samples collected with a collection device that stimulates OF production (Wille et al., 2009, Gjerde et al., 2010), as well as for those devices that utilise non-stimulated sample collection techniques (Vindenes et al., 2012a). Thus this relationship remains difficult to characterise and the objective ‘assessment of impairment from OF currently impossible’ (Vindenes et al., 2012a). Further discussion of OF: Blood drug ratios can be found below.

**4.4 The relationship between blood and oral fluid (oral fluid: blood ratios)**

Drummer et al suggests that if the intention is to detect the presence of psychoactive substances at a time when the driver has been apprehended and deemed to be a high
road safety risk, the use of OF would be problematic (Drummer et al., 2007). OF: blood ratios are known to vary from drug to drug, from person to person, and even intra-individually making efforts to relate OF drug concentration to certain equivalent blood drug concentrations very challenging (Gjerde et al., 2010, Wille et al., 2009). This is particularly the case if taking into consideration release of drugs from buccal depots. The many factors that control drug disposition from the general circulation are largely responsible for this variability. In theory, individual differences may arise as a circulating drug crosses different membranes (capillary, basal) and the epithelial cells of the salivary glands before it can pass into OF. In addition, highly sensitive methodologies such as chromatography with tandem mass spectrometry (MS/MS) would be essential for analytical purposes (Liu et al., 2015).

For evidential (confirmatory) testing purposes using a per se (threshold) approach, the usefulness of OF as a possible matrix would be dependent on consistent oral fluid-whole blood (OF: B) ratios. For OF concentrations to accurately predict whole blood concentrations, the OF: B ratio would need to be independent of drug concentration and consistent between individuals. Attempts have been made to establish fixed ratios or conversion factors between drug concentrations in blood and those in OF for confirmation testing. However, due to large individual variations, ratios have been difficult to agree and cannot be easily determined for most psychoactive drugs, although some correlation has been described (Cone et al., 1988, Wille et al., 2009, Walsh et al., 2004a, Walsh et al., 2004b). Drummer published the following table (Table 20) of OF: Blood ratios for the Australian Standards Forum (Drugs in Oral Fluid AS4760, Standards Australia Forum, 2006).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Number samples</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>158</td>
<td>16</td>
</tr>
<tr>
<td>Cocaine</td>
<td>40</td>
<td>6</td>
</tr>
<tr>
<td>MDMA</td>
<td>54</td>
<td>3.3</td>
</tr>
<tr>
<td>Morphine</td>
<td>17</td>
<td>2.7</td>
</tr>
<tr>
<td>THC</td>
<td>323</td>
<td>16 – 20</td>
</tr>
</tbody>
</table>
An alternative approach for the drug-driving context would be the use of OF with a laboratory-based cut-off (LLOQ or LOD). This would not need to be based on predicting blood concentrations because the goal would be to determine the presence or absence of a drug above a laboratory determined threshold. Thus OF: whole blood ratios would be of less importance (Desrosiers et al., 2013).

Detection and residence time of Individual drugs from section 5A of the drug-driving legislation including OF: whole blood ratios and other pertinent characteristics are discussed below:

### 4.4.1 Cannabis
THC is the predominant analyte detected in OF following cannabis use although there is some debate over whether the detection of the primary metabolite THC in OF is due primarily to contamination of the oral cavity or smoking or ingestion of the drug (see review by Wolff and Johnston (Wolff and Johnston, 2014). Some cannabinoids are highly protein bound and do not pass readily from blood into OF (Karlsson and Strom, 1988). However, increased THC OF concentrations may well be found in naso-oral cavities following smoking (Akrill and Mason, 2004) and release may occur from depots in the buccal mucosa.

Researchers have demonstrated a common pattern of detection in OF after smoking cannabis cigarettes. Usually initial very high and varied OF THC concentrations are measured. Concentrations ranging from 18 μg/L to 1080 μg/L (Niedbala et al., 2005) and 245 μg/L to 2544 μg/L (peak 5800 μg/L) immediately after smoking (Huestis and Cone, 2004) have been reported. Similar patterns of OF THC detection have been noted in occasional users ((397 μg/L - 6438 μg/L) and chronic users (387 μg/L - 71747 μg/L) after smoking a standardized cannabis cigarette (Toennes et al., 2010). THC then clears rapidly from OF within 1 – 3 hours (Lee et al., 2012). For instance, THC concentration fell noticeably after 8 hours to median of 6.3 μg/L (occasional) and 11.3 μg/L (chronic), respectively (Toennes et al., 2010), whilst Huestis and Cone (Huestis and Cone, 2004) reported a slow decline with THC OF concentrations <1 μg/L within 12 hours. In addition, Kauert et al., (Kauert et al., 2007) found OF THC concentrations were dose related with peaks of 900 ± 589 μg/L and 1041 ± 652 μg/L after smoking a 18 mg or a 36 mg THC cigarette.

With regards to cannabis metabolites conversion from THC to 11-OH-THC, the main active metabolite of THC is greater when cannabis is consumed in the form of edibles compared to when it is smoked (Moore et al., 2007b), but is usually only present at low concentrations (0.3 – 1.3 μg/L) in OF. THC-COOH has also been detected in OF. Using the
Quantisal™ (Immunalysis) device THC-COOH concentrations were reported after smoking to be 134 – 760 μg/L (Moore et al., 2007a), whilst a concentration of 560 μg/L was observed in expectorated OF (Milman et al., 2012). In OF collected with the Oral-Eze device for up to 30 hours after controlled smoking of a cannabis cigarette (6.8 % THC) by frequent and occasional smokers, frequent smokers had significantly greater OF THC-COOH concentrations than occasional smokers at all times (Newmeyer et al., 2014).

In terms of detection times in OF, with a cut-off of THC ≥1 µg/L the last detection time in the laboratory using GC-MS was between 1 hour and 13.5 hours if combined (same cut-off ≥1 µg/L) with the minor naturally occurring cannabidiol (CBD) or cannabinol (CBN). Cut-offs utilizing THC alone or combined with THC-COOH showed significantly different window of detection times between frequent and occasional smokers. The widest detection windows were observed with a OF THC cut-off ≥1 or 2 µg/L or THCCOOH ≥ 20 µg/L (Newmeyer et al., 2014).

Passive exposure to cannabis smoke inside an 8-man van did not give rise to false positive test results for THC in OF using the Intercept Oral Specimen Collection Device analysed using GC-MS, whereas in those exposed to passive cannabis smoke for 3 hour in a Dutch coffee-shop OF THC concentrations at or near 2 µg/L were detected (Niedbala et al., 2005, Niedbala et al., 2004, Moore et al., 2011). Factors such as room size and extent of THC smoke exposure clearly impact on test results.

Using the Intercept® collection device OF was collected following use of a standard cannabis cigarette and the median OF: serum ratio was 16 without any differences observed between chronic and occasional users (Toennes et al., 2010), which is at the lower end of the ratio range reported in Table 20 (Standards Australia Forum, 2006).

4.4.2 Cocaine and BZE

The relative acidity of OF compared to plasma, means that basic drugs such as cocaine are frequently found in OF in higher concentrations than in plasma, yielding OF: blood ratios greater than unity (Cone, 1993). Use by snorting or smoking will lead to increased cocaine concentrations in the oral cavity although these high concentrations appear to clear quickly. Studies indicate that cocaine concentrations fall rapidly after smoking and reach OF drug concentrations suggestive of consumption rather than contamination within 60 minutes (Crouch, 2005).

Most screening methods for cocaine are based on an immunoassay process and many will detect both cocaine and BZE in urine and in OF. Some are designed to be more specific for BZE. This is an attempt to recognise the longer detection time required when prior use of cocaine, rather than recent use of cocaine, is important. Most commercial OF cocaine
immunoassay screening tests cross-react appreciably with the major cocaine metabolites, which reduces test specificity and prolongs the window of detection.

The use of OF for the quantitative detection of cocaine for evidential purposes has been reported to be variable because different collection techniques can have a considerable influence on the concentration of drugs found in OF samples (Crouch, 2005, Crouch et al., 2005). The European ROSITA-2 project evaluated the usability and analytical reliability of the OF drug testing devices (Verstraete and Raes, 2006). Nine devices were evaluated for drugs including cocaine. Extensive variation was found in the OF concentrations of cocaine and BZE.

In a study by Wille (Wille et al., 2009) the mean OF: whole blood ratio for cocaine averaged 22:1 (range of 4:1 to 119:1) and the mean for BZE was 1:1 (range of 0.2:1 to 11:1). Whereas the DRUID (Deliverable 0.1.8, 2012) studies reported a mean OF: blood ratio for BZE of 2.82 (range 1.83 - 3.81, 95 % CI); for cocaine the mean was 20.5 (range 13.3 - 27.7, 95 % CI) (DRUID, 2012). Other OF: whole blood ratios have been published as shown in Table 21.
<table>
<thead>
<tr>
<th>Study details (Author)</th>
<th>Dose</th>
<th>Peak blood, serum or plasma concentration</th>
<th>Peak OF concentration</th>
<th>OF: blood, plasma or serum for cocaine</th>
<th>OF: blood, plasma or serum for BZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 6, *stimulated collection (Cone et al., 1997)</td>
<td>Cocaine: 25 mg IV 32 mg IN and 42 mg SM</td>
<td>Cocaine: 258 – 1303 μg/L (IV) 75 – 14743 μg/L (IN) 94 – 7737 μg/L (SM)</td>
<td>Cocaine: 1.3 to 10.1 (IV) 0.3 to 15.5 (IN) 0.4 to 5.2 (SM)*</td>
<td>0.1 to 0.8 (IV) 0.1 to 2.3 (IN) 0.1 to 0.5 (SM)</td>
<td></td>
</tr>
<tr>
<td>n = 6, *stimulated collection (Jufer et al., 2006, Jufer et al., 2000)</td>
<td>Cocaine: 25 mg IV 32 mg IN and 42 mg SM</td>
<td>Cocaine: 0.5 to 25.3^</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 68 (Schramm et al., 1993)</td>
<td>Used cocaine within last 24 h</td>
<td>Cocaine: 3.4 – 395 μg/L (0-8 h) 2.3 – 294 μg/L (8-16 h) 0.66 – 87 μg/L (16-24 h) Benzoylcegonine: 118 – 2470 μg/L (0 – 8 h) 16 – 3100 μg/L (8 – 16 h) 3.7 – 1000 μg/L (16 -24 h)</td>
<td>Cocaine: 3.7 – 1480 μg/L (0 – 8 h) 11 – 959 μg/L (8 – 16 h) 2.7 - 1990 μg/L (16 – 24 h) Benzoylcegonine: 10 – 1960 μg/L (0 – 8 h) 9 – 1910 μg/L (8 – 16 h) 8.8 – 363 μg/L (16 – 24 h)</td>
<td>OF: serum 3.85 (0 – 8 h) 3.45 (8 – 16 h) 20.0 (16 – 24 h)</td>
<td>OF: serum 2.0 (0 – 8 h) 1.19 (8 – 16 h) 2.17 (16 – 24 h)</td>
</tr>
<tr>
<td>n = 75, *stimulated collection (Kolbrich et al., 2006)</td>
<td>Cocaine: 75 and 150 mg/70 kg, SC</td>
<td>Cocaine: 1322 μg/L (70 mg) 3130 μg/L (150 mg)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 21  Published OF: Blood (plasma/serum) ratios for cocaine and BZE following different routes of administration

<table>
<thead>
<tr>
<th>Study details (Author)</th>
<th>Dose</th>
<th>Peak blood, serum or plasma concentration</th>
<th>Peak OF concentration</th>
<th>OF: blood, plasma or serum for cocaine</th>
<th>OF: blood, plasma or serum for BZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 7, *stimulated collection (Jenkins et al., 1995)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cocaine: 40 mg (SM) and 44.8 mg (IV)</td>
<td>Cocaine: 46 – 291 μg/L (SM) 122 – 427 μg/L (IV)</td>
<td>Cocaine: 15852 – 504880 μg/L (SM) 428 – 1927 μg/L (IV)</td>
<td>1.35 to 44.23 (SM) 0.90 to 7.9 (IV)</td>
<td>0.02 to 0.80 (SM) 0.02 to 0.66 (IV)</td>
</tr>
<tr>
<td>n = 20, *stimulated collection (Moolchan et al., 2000)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cocaine: 75 mg/70kg (n = 19) 150 mg/70kg (n = 14) SC</td>
<td>Cocaine: 109 – 434 μg/L (75 mg) 254 – 1154 μg/L (150 mg)</td>
<td>Benzoylecgonine: 180 – 411 μg/L (75 mg) 336 – 832 μg/L (150 mg)</td>
<td>Mean &gt;1 (0.5 – 24 h)</td>
<td>Mean &lt;1 (0.5 – 24 h)</td>
</tr>
<tr>
<td>n = 33, *stimulated collection (Scheidweiler et al., 2010)</td>
<td>25 mg (IV)</td>
<td>Cocaine: 356 – 14700 μg/L</td>
<td>Benzoylecgonine: 96 – 720 μg/L</td>
<td>Median &gt;1 (0.17 – 2 h)</td>
<td>Median &gt;1 (0.17 – 1 h)</td>
</tr>
<tr>
<td>n = 10 (Ellefsen et al., 2014)</td>
<td></td>
<td></td>
<td></td>
<td>Median c.1 (3 – 13 h)</td>
<td>Median &lt;1 (1 – 21 h)</td>
</tr>
</tbody>
</table>

After one hour, for benzoylecgonine the ratios were 0.1 to 0.8 (IV), 0.1 to 2.3 (IN) and 0.1 to 0.5 (SM); ^Benzoylecgonine serum concentrations were on average 2.5 times higher than those in OF. +Peak OF benzoylecgonine concentrations reported were from 10-1960 μg/L at 0 – 8 h, 9 – 1910 μg/L at 8 – 16 h and 8.8 – 363 μg/L at 16 – 24 h. The corresponding OF: serum ratios were 3.85, 3.45 and 20.0.
4.4.3 Ketamine
Whereas the secretion of saliva is reduced by many drugs, ketamine has a stimulatory effect on its production (Yew, 2015). Ketamine and its major metabolite norketamine have been detected by wooden-tip electrospray ionization mass spectrometry (WT-ESI-MS) in standard OF solutions (So et al., 2013). As yet however, commonly used OF POCT drug screening devices do not include ketamine. Stranno-Rossi et al (Strano-Rossi et al., 2012) found ketamine by chance in 2.3% of OF samples tested using OF POCT devices when confirming test results with UHPLC–MS/MS. Some ketamine specific POCT screening test kits are beginning to emerge. The OratectXP OF Drug Screen Device for ketamine (cut-off specification 15 µg/L) was evaluated and found to be suitable for roadside screening drug tests (Tsui et al., 2012).

4.4.4 LSD
LSD is not detected very often in biological samples although methods for its determination in OF have been described. Oiestrad et al (Oiestad et al., 2007) reported 68 – 82% extraction recovery from preserved OF collected using the Intercept® device and spiked with known quantities of LSD.

4.4.5 Opiates and Opioids
The presence of morphine in OF or urine can be indicative of either medicinal (diamorphine or morphine) or illicit (heroin) opioid use, whereas the presence of codeine alone in OF or urine is usually as a result of the legitimate consumption of anti-tissive or analgesic preparations (Hawks and Chiang, 1986).

Orally administered morphine shows a delay in its appearance in OF compared to its presence in plasma suggesting some rate limiting movement in OF possibly due to its relatively low lipid solubility (Drummer, 2006). OF when collected from heroin users has a high prevalence of 6-MAM, which is an advantage since its detection in urine occurs infrequently and detection in blood is limited due to its short half-life (Allen, 2011, Jenkins et al., 1995). Some metabolites of opioid drugs present in OF in high concentrations; the demethylated metabolites of oxycodone, hydrocodone, and codeine are present at higher concentrations in OF than oxymorphone, hydromorphone, and morphine, respectively; therefore, their detection in OF is also beneficial and indicates ingestion (Tuyay et al., 2012). However Hardy et al (Hardy et al., 2012) reports that measurements of both oxycodone and its major metabolite present in OF does not provide a valid substitute for plasma in pharmacokinetic studies.

During the ROSITA-2 Project blood and OF samples were collected from drivers stopped during random controls by the police in Belgium, Germany, Finland, and Norway and the
OF: blood (OF: B) ratios were calculated for various drugs including the opioids (Verstraete and Raes, 2006). The ratios found in this study were comparable with those that were published previously, but the range was wider (Wille et al., 2009).

It was concluded that the variability of the OF: Blood ratios does not allow reliable estimation of the blood concentrations from OF concentrations in drivers thought to be under the influence of opioid drugs. This is particularly the case if a per se approach were to be used for evidential purposes. OF would be difficult to introduce because of the complicated matter of determining if the individual was impaired due to recent drug ingestion; only a blood sample can confirm this satisfactorily (Standards Australia Forum, 2006).

4.4.5.1 Methadone
In the European Driving Under the Influence of Drugs, Alcohol and Medicines Study (DRUID) methadone was readily identified in OF at concentrations >20 µg/L (the cut-off set by the DRUID (DRUID, 2012), following daily 30 – 110 mg/day methadone pharmacotherapy. All OF specimens contained methadone concentrations >1 µg/L; 88 % were positive for the main metabolite EDDP and 12 % positive for the secondary metabolite methadol; neither metabolite was detected alone. Methadone and EDDP OF concentrations were highly variable within and between participants (Gray et al., 2011). The correlations between methadone in OF and plasma (r = 0.46) and OF and blood (r = 0.52) has also been determined and the mean OF: plasma and OF: blood distribution ratios were calculated to be 0.55 and 0.77, respectively. The methadone concentration in OF decreased as salivary pH increased (Hsu et al., 2013).

4.4.6 Amphetamines
Weak bases such as the amphetamines tend to concentrate in OF because of the more acidic environment compared to plasma; the parent drug is usually the main compound found in this matrix. However buccal contamination from orally or nasally administered drugs cannot be ruled out. A further complication occurs with methamphetamine; d-methamphetamine is a scheduled controlled stimulant, while l-methamphetamine is the unscheduled active ingredient in over-the-counter nasal decongestant Vicks® VapoInhaler™, available in North America. Newmeyer et al (Newmeyer et al., 2015) established that methamphetamine can be detected in OF and plasma after Vicks® VapoInhaler™ administration and concluded that chiral chromatography would be necessary to rule out VapoInhaler™ intake.
4.4.7 Benzodiazepines

For benzodiazepine drugs, the parent compounds are often found in OF whereas only their metabolites are be found in urine (Allen, 2011). However, it is widely acknowledged that detection of benzodiazepines in OF is especially challenging owing to low concentrations due to low therapeutic dosing (e.g., clonazepam), high plasma protein binding (Jang et al., 2013), high lipophilicity and analyte instability in non-preserved OF (Bosker and Huestis, 2009). Poor sensitivity has been reported for benzodiazepines with on-site OF POCT screening tests, including Rapid STAT®, DrugWipe5/5+® and the Dräger DrugTest® 5000 (Gronholm and Lillsunde, 2001, Musshoff et al., 2014).

Of the drugs included in section 5A of the new legislation; Speedy et al (Speedy et al., 2007) observed a good recovery (92.1 %) from the Cozart DDS® collection device for temazepam and, Quintela et al (Quintela et al., 2006) reported a recovery of 101.3 % for oxazepam with the Immunalysis Quantisal collection device. Flunitrazepam was detected in expectorated OF after oral dosing of 1 mg for only 6 hours. Concentrations were <0.6 μg/L unless OF was preserved with 2 % NaF (Samyn et al., 2002a).

In a roadside survey as part of the DRUID study OF was collected using the Statsure collection device in Hungary (n = 2738). Confirmation was carried out using GC-MS. Diazepam was the most common medicinal drug detected (OF concentration range 4.88 - 53.7 μg/L) followed by clonazepam (OF 2.24 – 135 μg/L). It should be noted that for both drugs the primary metabolite was detected more often than the parent drug (nordiazepam on 33 occasions compared to 9 for diazepam and on 23 occasions for 7-amino clonazepam compared to 4 for clonazepam (Institóris et al., 2013). Melanson et al has recently reported that 7-amino clonazepam is superior to clonazepam for detection of clonazepam use in OF (Melanson et al., 2016).

Investigators reported that after 15 or 30 mg oxazepam, oxazepam and oxazepam glucuronide were detected in the expectorated OF of subjects, for more than 8.5 hours, in much lower concentrations than in simultaneously collected blood. OF: blood ratios ranged from 0.04 to 0.07 for oxazepam and from 0.002 to 0.006 for oxazepam glucuronide (Smink et al, 2008).

4.5 Commercial devices used for OF drug testing

Over a decade ago (between 2003 and 2005), in recognition of the growing use of OF as a drug screening matrix, a large European study (the ROSITA-2 project) was carried out. Funded by the National Institute on Drug Abuse (NIDA), National Institute of Health, the National Highway Traffic Safety Administration (NHTSA), the Office of National Drug Control Policy and the Executive Office of the President, the ROSITA-2 project sought to
evaluate the usability and analytical reliability of several onsite OF POCT drug screening devices (Raes and Verstraete, 2005, Verstraete and Raes, 2006).

Nine devices were evaluated (Table 22) and were used to test for the following drugs; amphetamines, methamphetamine, cannabis, cocaine and opiates. Subjects for whom a suspicion of driving under the influence of drugs existed were invited to participate in the study. In most cases a blood sample and an OF sample were collected; OF with the Intercept® OF sampler (Orasure Technologies Inc, 2016). Analysis took place in the laboratory using GC or LC-MS.

The percentage of positive samples collected in the ROSITA-2 studies were; amphetamine (including methamphetamine, ecstasy and analogues) 20 %, benzodiazepines 32 %, cannabinoids 36 %, cocaine 19 % and opiates 8 %. The analytical evaluation of the amphetamine-type tests (in comparison to GC-MS in OF) showed a sensitivity (percentage of the true positive samples that tested positive with the onsite assay) varying between 40 % and 83 % and a specificity (percentage of the negative samples that tested negative with the onsite assay) between 80 % and 100 %. The analytical evaluation of the benzodiazepine tests showed a sensitivity varying between 33 % and 69 % and a specificity between 85 % and 94 %. The performance of the onsite OF tests were wide ranging and not particularly sensitive for benzodiazepines (Verstraete and Raes, 2006).

Table 22  POCT drug testing devices used in ROSITA-2 (Verstraete and Raes, 2006)

<table>
<thead>
<tr>
<th>POCT Devices</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cozart® RapIScan Bioscience (USA)</td>
<td><a href="http://www.solmazbilmed.com/Docs/Cozart-Rapiscan.pdf">http://www.solmazbilmed.com/Docs/Cozart-Rapiscan.pdf</a></td>
</tr>
<tr>
<td>Sun Biomedical OraLine®</td>
<td><a href="https://www.americanscreeningcorp.com/training/OraLine_I_V_Insert_Forensic_Use_Printable.pdf">https://www.americanscreeningcorp.com/training/OraLine_I_V_Insert_Forensic_Use_Printable.pdf</a></td>
</tr>
<tr>
<td>Varian OraLab™,</td>
<td><a href="http://www.yarebio.cn/Varian/oralab/index.htm">http://www.yarebio.cn/Varian/oralab/index.htm</a></td>
</tr>
</tbody>
</table>
In a further study 10 POCT OF drug testing devices including Oralstat (American Bio Medica); SmartClip® (EnviteC, 2016); Impact (Business Wire, 2004); and OraLine® IV s.a.t (Drugcup, 2016) were compared for their ability to meet the claimed (and proposed) cut off concentrations set by the manufacturers for the detection of amphetamine(s), cocaine/BZE, opioids, cannabinoids and Oralstat for benzodiazepines. Most devices performed well for the detection of opioids and amphetamine(s), but approximately half had amphetamine(s) cut-off concentrations greater than recommended by SAMHSA. Only three devices had cocaine cut-offs ≤20 µg/L (SAMHSA), and a number of false-negative results were obtained (Walsh et al., 2007). Others have reported that the DrugWipe® 5/5 did not always perform well for compounds such as cannabis, opiates and cocaine (Pehrsson et al., 2011).

Commercial devices used to detect specific compounds in OF are discussed below:

4.5.1 Cannabis
THC has traditionally been a difficult drug to analyse using immunoassay techniques. The analytical evaluation of THC OF immunoassay tests in comparison to OF chromatographic methods showed a sensitivity varying between 0 % and 74 % and a specificity between 70 % and 100 %. Detailed analysis of the data for cannabis showed that some devices e.g., DrugWipe® gave a negative result even when very high concentrations of THC were found with the Intercept collection device (Verstraete, 2005, Verstraete and Raes, 2006). The reason for these differences was unknown, but it was acknowledged that an improved (more thorough) sampling technique could capture more THC, resulting in better sensitivity (true positives). Nine years ago none of the 10 OF devices that Walsh et al employed were capable of detecting THC at the SAMHSA cut-off of 4 µg/L (Walsh et al., 2007) and the OF testing devices were not considered to be reliable enough to be recommended for roadside screening of drivers.

However significant technological improvements in analytical methods including; more sensitive OF POCT immunoassays; improvements in the sensitivity of laboratory equipment through the use of LC-MS/MS and; new sample processing procedures using low sample volumes, has enabled the expansion of the OF THC drug test portfolio (Moore et al., 2011). It was concluded by the Panel that the sensitivity and performance of commercial OF drug testing devices had noticeably improved although not all achieved the standards required for the reliable detection of THC, especially at low concentrations (Table 23). There are two POCT immunoassay devices, type-approved with sensitivity and specificity parameters suitable for use in the UK to screen for THC; the Dräger DrugTest®

13 There are several different models of the DrugWipe product but only one has been type-approved for use in the UK as described on page 95
5000 (Dräger UK, 2016) and the DrugWipe-2® (Securetec, 2016). The Dräger Drug Test® (DDT) 5000 and the DrugWipe-2® devices currently employed by police officers for roadside screening have a cut-off for THC of 10 µg/L.

4.5.2 Cocaine and BZE

A major problem with cocaine use and sample volume is that the drug reduces salivation (inducing a dry-mouth), a problem common to many stimulant drugs (Fratto and Manzon, 2014). Kato et al (Kato et al., 1993) investigated the difference between stimulated and non-stimulated OF collection in 6 subjects administered a 25mg IV dose of cocaine hydrochloride and demonstrated that the latter contained substantially more cocaine. Other researchers have found different results. Scheidweiler et al (Scheidweiler et al., 2010) found no difference in cocaine concentrations in OF collected by stimulated expectoration and a neutral cotton Salivette® collection device.

The Salivette® collection device has been used to collect OF as an alternative specimen to urine for monitoring illicit substances (Dams et al., 2007). Recoveries from the Salivette® between 70 % and 85 % of OF volume and mean recovery of cocaine and BZE of 81.7 to 91.4 % were observed whereas mean recovery from the Draeger DCD 5000 OF collection device was better and reported to be 95.6 % and 86.8 % for cocaine and BZE respectively (Hall et al., 2015, Vanstechelman et al., 2012).

There are two POCT immunoassay devices, type-approved with sensitivity and specificity parameters suitable for use in the UK to screen for cocaine and BZE; the Dräger DrugTest® 5000 (Dräger UK, 2016) and the DrugWipe-2® (Securetec, 2016). The Dräger Drug Test® (DDT) 5000 and the DrugWipe-2® devices currently employed by police officers for roadside screening have a cut-off for cocaine of 30 µg/L.
<table>
<thead>
<tr>
<th>Screening Device</th>
<th>Country use / Manufacturer</th>
<th>Sensitivities</th>
<th>Specificity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>THC</td>
<td>Cocaine</td>
<td>Amphet</td>
</tr>
<tr>
<td>Dräger DrugTest® 5000</td>
<td>Luebeck, Germany</td>
<td>87%</td>
<td>76%</td>
<td>84%</td>
</tr>
<tr>
<td>(Dräger Safety AG &amp; Co. KGaA</td>
<td></td>
<td>81%</td>
<td>50%</td>
<td>75%</td>
</tr>
<tr>
<td>Innovacon OrAlert</td>
<td></td>
<td>23%</td>
<td>50%</td>
<td>33%</td>
</tr>
<tr>
<td>Cozart Rapiscan Cozart DDS 806</td>
<td>UK</td>
<td>28%</td>
<td>11%</td>
<td>67%</td>
</tr>
<tr>
<td>DrugWipe5/5** [DrugWipe*]</td>
<td>Brunnthal, Germany</td>
<td>71%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>[DrugTest® 5000]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapid STAT® (Mavand Solution GmbH)</td>
<td>Mössingen, Germany</td>
<td>91%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>43%</td>
<td>27%</td>
<td>77%</td>
</tr>
</tbody>
</table>

*Different authors have reported different test results for a single device; ** Specificity has been reported to differ for different models of the same device.
4.5.3 Opiate and Opioids

For opioid drugs, older immunoassay tests for morphine tended to cross-react with codeine, 6-MAM, morphine-3-glucuronide and morphine-6-glucoronide (Litman et al., 1983). Consequently, if more than one of these substances is present in a biological fluid the test result would relate to the concentration of the sum of all these drugs and their metabolites (Wolff, 2006).

Illicit use of heroin or medicinal diamorphine may be identified by the detection of 6-monoacetylmorphine (6-MAM). Specific immunoassays available for 6-MAM such as from the Immunalysis Corporation (immunalysis.com) and from Siemens (Borriello et al., 2015) are now available on the market. The former is a homogenous enzyme immunoassay with a cut-off of 10 µg/L in urine. The specificity of the test screen is summarized in Table 24.

Table 24 Cross reactivity of the Immunalysis test for 6-MAM (expressed as the minimum concentration of metabolite or compound required to produce a response equivalent to the cut-off of the assay (immunalysis.com)).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Conc. (µg/L)</th>
<th>% Cross-Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-Monoacetylmorphine</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>6-Acetylcodcidne</td>
<td>600</td>
<td>1.7</td>
</tr>
<tr>
<td>Diamorphine</td>
<td>1,375</td>
<td>0.7</td>
</tr>
<tr>
<td>Hydromorphone</td>
<td>325,000</td>
<td>0.003</td>
</tr>
<tr>
<td>Morphone</td>
<td>285,000</td>
<td>0.0035</td>
</tr>
<tr>
<td>Nalorphine</td>
<td>80,000</td>
<td>0.0125</td>
</tr>
<tr>
<td>Naloxone</td>
<td>300,000</td>
<td>0.0033</td>
</tr>
<tr>
<td>Naltrexone</td>
<td>390,000</td>
<td>0.0026</td>
</tr>
<tr>
<td>Normorphine</td>
<td>250,000</td>
<td>0.004</td>
</tr>
<tr>
<td>Oxymorphone</td>
<td>360,000</td>
<td>0.0028</td>
</tr>
</tbody>
</table>

No cross-reactivity was observed for the following drugs (not detected by the immunoassay at a concentration of 1,000,000 µg/L); buprenorphine, codeine, dihydrocodeine, dextro methorphan, ethylmorphine, hydrocodone, levorphanol, meperidine, morphine 3-D glucuronide, naproxen, norcodeine and oxycodone.

There is no POCT device that can detect all opioids and their metabolites in either urine or OF; some opioids are challenging even for the fully equipped toxicology laboratory.
(Table 23). For instance, whilst in some European countries 6-MAM and tramadol are the most commonly found opioids in OF donated by impaired drivers; not all immunochemical screening can detect tramadol. General screening tests for opioids are also unlikely to show a positive result for oxycodone unless the concentration of the drug is very high (DRUID, 2012).

The Siemens EMIT II Plus 6-AM (6-MAM) urine assay has been modified and validated to qualitatively screen for 6-MAM in OF using an OF calibrator at the SAMHSA cut-off concentration of 4µg/L with the dilution and buffering system of the Quantisal™ OF collection device (Alere™, 2016b).

Newer immunoassay tests are available for medicinal opioids (Milone, 2012). Nevertheless an immunoassay screening test that is positive for opiates could be the result of several different circumstances of opiate consumption. Both EMIT ® (Fraser and Worth, 1999) and Abuscreen Radioimmunoassay (RAI) detect codeine and morphine in free and conjugated (glucuronide) forms but these tests do not distinguish between them (Boettcher et al., 2000). Immunoassay screening tests used for OF or urine usually therefore serve only as a guide, and results would need to be confirmed by using another methodology (Karch and Drummer, 2001).

4.5.4 Amphetamines
Amphetamine can be easily detected in OF using on-site POCT tests (Engblom et al., 2007), although cross-reactivity with other amphetamine-type substances has been a problem in this matrix especially if earlier devices were used. Current OF POCT drug screening devices perform well for amphetamines, with diagnostic sensitivities, diagnostic specificities, and efficiencies in the range of 70% – 100 % (Bosker and Huestis, 2009). Whereas, diagnostic sensitivity has been reported to be low for the Securetec DrugWipe® (Crouch et al., 2008), the Varian OraLab® and the Dräger Drug Test®-DDT (Concheiro et al., 2007). A comparison of the performance of several screening devices can be found in Table 23.

Two different multi-drug OF screening devices (OFDs) (Screen® Multi-Drug OFD and GIMA One Step Multi-Line Screen Test OFD) targeting amphetamine or methamphetamine were evaluated to determine the cross-reactivity of thirty-nine new amphetamine-type ‘designer’ drugs. Only two compounds (p-methoxyamphetamine and p-methoxymethamphetamine) of all the amphetamines tested gave a positive result (Nieddu et al., 2014).
4.5.5 Benzodiazepines

The DrugWipe® Benzodiazepine Single Device (Securetec) has been reported to detect benzodiazepines in driving under the influence of drugs (DUID) cases including (as confirmed by GC-MS) diazepam, nordiazepam, oxazepam, temazepam and clonazepam (Blencowe et al., 2011).

4.6 POCT PERFORMANCE CHARACTERISTICS

The Panel noted the variability in the performance of roadside POCT drug screening devices with wide ranges in sensitivity and specificity for the both illicit and medicinal drugs. The lack of consistency in performance of screening devices has been reported by a number of researchers in different conditions and with differing subject populations (Table 23).

Comparative data on different POCT screening tests helps take the field forward. In a prison population the performance of the Dräger Drug Test® (DDT) 5000 (Dräger UK, 2016) and the DrugWipe® (Securetec, 2016) POCT devices using urine or OF were compared. Overall sensitivities of 51 % and 53 % were achieved for all drugs tested and positive predictive values of 93 % and 63 %, respectively. Both devices failed to detect benzodiazepines (although as noted above the more specific DrugWipe® Benzodiazepine Single Device performs well). The Dräger Drug Test® DDT 5000 had better sensitivity in detecting THC use (Jones et al., 2014).

The sensitivity, specificity and accuracy of OF screening devices is a key consideration when using POCT devices in the drug-driving context. The Panel endorsed the need for a minimum standard specification for commercial tests, to include the sensitivity and specificity of the device such that false positives were kept to an agreed minimum. This has been achieved in the UK by using a type-approval process.

Minimum standards have also been established in the DRUID studies and was set at 80% for each parameter (DRUID, 2012). A study in Italy demonstrated that the Dräger DrugTest (DDT) 5000® fulfilled the DRUID criteria for all drug classes (Strano-Rossi et al., 2012). The State of Victoria (Australia) has produced ‘Performance Guidelines’ for POCT OF testing devices, which requires drug test accuracy of ≥ 95 %; sensitivity of ≥ 90 % and; specificity of ≥ 90 %. These performance guidelines were endorsed in the ROSITA studies (Verstraete and Raes, 2006).

4.6.1 Innovations

The number of commercially viable POCT tests developed for drug screening applications has increased (Slowey, 2013) alongside other innovative approaches. POCT drug testing
using OF from medical swabs appears to be competitive with that of screening immunoassays in respect of the time required for analysis of short acting drugs such as ketamine (Pirro et al., 2015).

### 4.6.2 POCT devices for evidential testing

The improvements with POCT devices has led to OF being used as an evidential matrix in drug driving in one State in Australia (State of Victoria) and in Spain. This is possible because some sampling systems retains the excess OF aliquot after screening. For instance, the Dräger DrugTest (DCD) 5000® is a twin OF collection device that allows the simultaneous sampling of two aliquots of authentic OF (Strano-Rossi et al., 2011).

The State of Victoria has a random roadside drug-testing programme and conducted 300,000 preliminary OF tests between 2005 and 2014. Drivers were tested at a roadside mobile laboratory for methamphetamine, MDMA and THC using the DrugWipe® (Securetec, 2016) screening tool. Following a positive test result with the DrugWipe®, a second OF sample was collected for confirmation using the OF Cozart® Rapiscan (Alere™, 2016a). If the second screening test (Cozart) was positive, half of the collected sample would be sent to a laboratory for evidential verification; where LLOQ is applied as the cut-off (ACE-P, 2016). First time offenders were issued with a penalty notice (fine and suspension of driving license) and repeat offenders were prosecuted (WHO, 2016, Boorman and Owens, 2009). In Australia however, the balance between the deterrent effect of random testing at the roadside and the knowledge that drivers may have a false negative test despite drug-driving due to the limited sensitivity of the screening test, has not been fully resolved (Boorman and Owens, 2009).

In Spain, a dual approach is used. Drug-driving is considered to be an administrative infringement, whereby any amount of drug present in the body is punishable with a fine (zero tolerance approach). Drug driving is also a criminal offence when a driver is shown to be impaired due to the influence of drugs and penalties include disqualification and imprisonment. Testing is carried out using roadside POCT OF screening and if that test is positive, a second mandatory evidential OF sample is collected and sent to the laboratory for confirmation (WHO, 2016, Fierro et al., 2015). Further information on international drug-driving policy and practice can be found in section 11.

### 4.7 OF SAMPLE COLLECTION SYSTEM/TUBES

Although in principle OF is easily obtained, there are often practical difficulties due to individual variability in salivation, particularly where a specific volume of OF is required. The Panel estimated that for laboratory-based evidential testing a minimum of 2 mL of OF would be required (double this amount if a sample were also to be made available to
the defendant) to measure drugs (n = 17) from differing drug classes. Two milli-litres is several fold the amount required for immunoassay POCT screening tests and may pose some difficulty for those with poor rates of salivation (as previously discussed).

The use of uncontrolled sample collection procedures may lead to variability in the detection of psychoactive substances and as a consequence drug use may be underestimated. For instance, the mean concentration of codeine is 3.6 times higher in specimens collected using a non-stimulated passive method compared to collection after acidic stimulation (Crouch et al., 2005). Similarly, reduced concentrations of cocaine, BZE and ecgonine methyl ester are found following stimulated OF collection (Kato et al., 1993). The Panel recommend that sample collection procedures will need to be taken into consideration if OF is to be used for confirmatory testing purposes.

Langel et al (Langel et al., 2008) investigated the suitability of 9 different OF collection tubes/systems for drug analysis and recorded variability in the recovery of different drugs from each collection system (Table 25). All of the collection systems enabled collection within 5 minutes using healthy volunteers as subjects (non-drug users). Some systems, such as the SCS (Greiner Bio-One Ltd, 2016), had a method of OF stimulation using citrate buffer whilst others, collected non-stimulated OF. It was found that collection systems varied in their suitability for drug testing purposes, a few e.g. OraCol (Malvern Medical Developments, 2016), and the Salivette® (Sarstedt AG, 2016) did not perform as well, particularly for THC and cocaine (Table 25).

It is clear from Langel’s study that there are important differences between commercial OF collection systems. Buffer solutions are varied and may help to increase the recovery of the drugs and improve the stability of the sample but, some may cause problems in achieving quantitative results since they may also contaminate the analytical equipment (Langel et al., 2008).

Using the Intercept® collection system morphine, 6-MAM, codeine, buprenorphine and methadone were identified using LC-MS/MS (positive ion mode electrospray) methodology to conduct road-side surveys in Norway (Øiestad et al, 2007). Extraction recoveries were >50 % except for morphine (30 %) and BZE (0.2 %). The concentrations of the lowest standard solution were 0.28 µg/L to 68 µg/L depending on the drug. In a different study again with the Intercept® OF collection system (Orasure Technologies Inc, 2016), a number of benzodiazepines were identified including alprazolam, bromazepam, clonazepam, diazepam, N-desmethyldiazepam, flunitrazepam, 7-aminoflunitrazepam, lorazepam, nitrazepam, oxazepam and the z-drugs (zopiclone, and zolpidem).
Table 25  Different types of OF sample collection tubes/systems adapted from Langel et al (Langel et al., 2008) and Verstraete et al (verstaete, 2011) with the percentage recovery for THC and Cocaine compared to a reference solution (n = 6).

<table>
<thead>
<tr>
<th>Collection device</th>
<th>Manufacturer</th>
<th>Description of device</th>
<th>Preservative/Buffer</th>
<th>Volume collected</th>
<th>Recovery THC from device (%)</th>
<th>Recovery cocaine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantisal saliva collection system</td>
<td>Immunalysis, Pomona, CA</td>
<td>Absorptive cellulose pad, polypropylene stem and plastic tube with buffer solution. Collection pad is placed under the tongue. Pad is placed in collection tube.</td>
<td>The volume of buffer solution is 3 mL, Contains a non-azide preservative.</td>
<td>1 mL ± 10% of OF is collected. Collection pad has volume adequacy indicator based on a blue dye. OF migrates along the cellulose pad by capillary action.</td>
<td>55.8 ± 12.0</td>
<td>81.7 ± 6.1</td>
</tr>
<tr>
<td>StatSure™ Sampler</td>
<td>StatSure Diagnostic Systems, Framingham, MA</td>
<td>Absorptive cellulose pad Collection pad placed under the tongue. Pad placed in bottom of the tube, with filter to recover the OF-buffer solution.</td>
<td>The volume of buffer solution is 1 mL. Yields a 1:2 v/v OF dilution</td>
<td>1 mL of OF is collected. Collection pad has a volume adequacy indicator (window in the stem of the collection pad turns blue).</td>
<td>85.7 ± 7.0</td>
<td>85.6 ± 4.9</td>
</tr>
<tr>
<td>Cozart Laboratory Screening Kit</td>
<td>Cozart (Alere™, 2016a)</td>
<td>Collection pad. Collector actively swabs gums, the tongue, and inside of cheek, then swab held inside the mouth until indicator turns blue.</td>
<td>The volume of the buffer solution is 2 mL. Buffer solution contains salts, preservatives, detergents.</td>
<td>The volume indicator area in the stem of the collection pad turns blue once 1 mL of OF has been collected.</td>
<td>75.9 ± 6.2</td>
<td>76.3 ± 4.2</td>
</tr>
</tbody>
</table>
Table 25: Different types of OF sample collection tubes/systems adapted from Langel et al (Langel et al., 2008) and Verstraete et al (verstaete, 2011) with the percentage recovery for THC and Cocaine compared to a reference solution (n = 6).

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</tr>
</thead>
<tbody>
<tr>
<td>Intercept oral specimen collection system</td>
<td>OraSure Technologies, Bethlehem, PA</td>
<td>Absorbent cotton fibre pad. Pad rubbed between lower cheek and gums until moist. The pad is held still for 2 – 5 min.</td>
<td>Preservative (0.8 mL); Flag Blue dye chlorhexidine digluconate, Tween 20 and deionised water.</td>
<td>37.6 ± 9.0</td>
<td>96.9 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>Greiner BiD-One Saliva Collection System</td>
<td>Greiner Bio-One GmbH, Kremsmünster, Austria</td>
<td>OF is collected by rinsing mouth with extraction solution (4 mL) containing citrate buffer to increase salivation. The extracted OF collected into the collection beaker.</td>
<td>OF transferred to 2 OF vacuum transfer tubes that contain stabilizers and preservatives.</td>
<td>73.6 ± 4.3</td>
<td>98.0 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>OraCol test kit</td>
<td>Malvern Medical Developments, Worcester, U.K</td>
<td>Absorbent foam swab. OF collected by rubbing the sponge firmly along the gum at the base of the teeth until the sponge swab is wet.</td>
<td>None stated Swab placed into the collection tube, shipped to laboratory, centrifuged to extract the sample from the swab.</td>
<td>Below cut-off &lt;12.5 %</td>
<td>35.1 ± 3.1</td>
<td></td>
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Table 25  Different types of OF sample collection tubes/systems adapted from Langel et al (Langel et al., 2008) and Verstraete et al (verstraete, 2011) with the percentage recovery for THC and Cocaine compared to a reference solution (n = 6).

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</tr>
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<tbody>
<tr>
<td>Salivette®</td>
<td>Sarstedt AG &amp; Co., N~mbrecht, Germany</td>
<td>Cotton swab in a plastic tube with an insert and a cap. Swab is placed into the mouth and chewed for approximately 45 s.</td>
<td>Plain swab or with a citric acid preparation</td>
<td>Swab centrifuged in the laboratory and the insert with the swab is removed to recover 0.5 – 2 mL clear OF</td>
<td>Below cut-off &lt;12.5 %</td>
<td>33.3 ± 8.7</td>
</tr>
<tr>
<td>OnTrack OraTube®</td>
<td>Varian, Lake Forest, CA</td>
<td>Foam collection pad. Foam collector is kept in the mouth until it is thoroughly soaked ≈ 3 min</td>
<td>The sponge contains an acid (with salty taste) that stimulates salivation</td>
<td>Designed to collect up to 1 mL. Foam collector pushed to bottom of the expresser so OF flows to the bottom of the tube.</td>
<td>47.5 ± 8.0</td>
<td>86.7 ± 3.3</td>
</tr>
<tr>
<td>Salicule saliva sampler</td>
<td>Acro Biotech, Rancho Cucamonga, CA</td>
<td>Vial with an expectoration straw. OF is expectorated via the straw into the vial until enough fluid is collected.</td>
<td>Not given</td>
<td>There is a scale on the side of the vial to note when full</td>
<td>45.9 ± 10.9</td>
<td>96.8 ± 2.8</td>
</tr>
</tbody>
</table>
Within- and between-day relative CVs varied from 2.0 % to 31.8 % and from 3.6 % to 39.1 %, respectively (Marin et al., 2012). There clearly remains issues with variability in the recovery (extraction of drug from the OF buffer-matrix solution and sampling equipment) and the detection of some drugs of interest using commercial OF collection devices (Valen et al., 2016).

If OF is to be collected using a commercial sampling system for evidential purposes then the recovery of the analytes of interest and the overall reliability of the collection procedure would need to be well characterised and a minimum standard established for those used at the road-side or in the police station. It is noteworthy that expectoration directly into a plastic tube recovered more THC than the use of a collection pad, swab or straw (Table 25). Moore and Crouch (Moore and Crouch, 2013) indicate that poor recovery may also be due to saturation when the collection pad is overloaded by researchers with unrealistic concentrations or when insufficient time is allowed for pads/swabs to remain in the transportation buffer. The impact of the OF collection sampling system on the whole toxicological procedure should not be underestimated.

4.7.1 OF Collection volume
The quantification of drugs in OF is hampered if specimen volume is not known, or is too small. Some of the currently available sample collection systems do not give an indication of how much OF is collected (Table 25), thereby rendering any quantification of results invalid without further manipulation in the laboratory (Moore and Lewis, 2003, Moore et al., 2007a, Kauert et al., 2006).

The amount of OF volume obtained varies both within and between devices. An evaluation of 3 collection devices provided within and between collection device tube volume variability ranging from 1.045 – 1.667 g OF (Dickson et al., 2007). This variability reflects imprecision in the elution buffer volume included in the sample collection kit, and more importantly, inconsistency in the amount of OF collected from the donor (Bosker and Huestis, 2009). The Orasure Intercept® sample collection tube/system was reported to collect from 0.38 – 1.53 g of OF (Bosker and Huestis, 2009). In addition, sampling systems sometimes incorporate a pad, swab or cotton bud for OF collection and do not always indicate how much OF is recovered from this sorbent material before analysis.

The Panel agreed that since the drug concentration reported is dependent on the sample collection procedure, it would be important to have some measure of the volume of OF required, in order to determine if a drug in OF is above the cut-off concentration for confirmatory purposes. For example, the Cozart® RapiScan (CRS)\textsuperscript{14} has a sample adequacy

\textsuperscript{14} At the time of the study Cozart was owned by Biosciences, UK
indicator in the plastic handle that turns blue when 1 mL of OF has been collected. The OF-soaked collection pad is mixed with 2 mL of Cozart proprietary buffer giving a final 1:3 dilution of the OF (Cooper et al., 2005). Some manufacturers provide quality assurance of variability in OF collection devices. Within-device variability of <10 % for the Quantisal collection device and <5 % for the Cozart and StatSure devices have been reported (Langel et al., 2008).

It is now standard practice for workplace drug testing programmes using OF that drug recovery from collection systems is verified by an accredited laboratory (SAMHSA, 2015, EWDTS, 2015). Recovery data are used in conjunction with collection volume imprecision data and uncertainty of measurement to provide an estimation of drug concentration in neat fluid. Hall and colleagues (Hall et al., 2015) calculated that the mean collection volume of the Dräger 5000 collection device swab was 487 µL (collection volume of swab according to manufacturer’s information, 380 µL) with an imprecision of 1.3 %. Recovery of drugs from the swab ranged from 86 % to 98 % for drugs listed in the Australian OF workplace drug testing standard (Standards Australia Forum, 2006).

In the drug-driving environment the Panel felt that an essential requirement would be, that the person collecting the OF sample should know that a sufficient sample has been collected, before subjecting the sample for laboratory testing.

### 4.8 Stability of Drugs in OF

There is generally a lack of information on the stability of illicit drugs in OF during transportation and storage until analysis. Efforts have largely concentrated on stability in the laboratory and in blood and urine (Peters, 2007). Ventura et al (Ventura et al., 2009) evaluated the stability of illicit drugs in OF together with their recovery from two collection systems typically used to ship OF samples to testing laboratories. Two different samples were prepared using the Cozart Drug Detection System (Alere™, 2016a) and the Intercept® OF collection system with 600 µg/L of 6-MAM and cocaine; 240 µg/L of THC and THC-COOH. Samples were sent at ambient temperature by courier to the participating laboratories (n = 19) the same day of preparation. Samples were analysed upon reception (48 – 72 h after shipment). Percent coefficients of variation (CV %), were around 30 % for all analytes, except for THC-COOH in both samples, which varied between 50 % and 80 %. On average, 9 – 12 % of 6-MAM was converted to morphine between sample collection and analysis and between 26 % and 41 % of cocaine to BZE. Good recoveries were observed for the THC-COOH metabolite of THC in both sample collection systems, whereas THC was always scarcely recovered.
The stability of drugs in OF before laboratory analysis is an important consideration in situations where the sample will not immediately be available to the laboratory. Two collection systems, the Intercept® (Orasure Technologies Inc, 2016) and the Saliva Sampler™ (SSUR, 2016) were tested using pools of authentic OF samples containing various drugs. The tests showed that 6-MAM, cocaine and zopiclone (not at present controlled in Section 5A) were the least stable compounds. In tests for short term stability, the Saliva Sampler® showed better results. This is an important consideration for re-analysis, which may be required in forensic situations. Tests after 1 year of storage at -20 °C showed that most of the compounds were stable for both sample collection systems with the exception of 6-MAM, cocaine and zopiclone (Lund et al., 2011).

More specific details of the stability of the drugs included in the section 5A drug driving legislation are given below with regards to OF (Table 26):

### 4.8.1 Cannabis

Many studies have been conducted to consider the stability of THC in OF using a sample collection tube/system. A pad, bud, swab or foam absorbent material is usually employed to collect an OF sample (Table 25). Following collection, which may be stimulated (by rubbing the material against the gums or by using pre-treated material) or passive (material is stationary in the buccal cavity), the OF is usually placed in a buffer solution for transportation to the laboratory. At the testing facility, a separator (tube or other device) is used to release the maximum amount of OF from the collection material into the buffer for analysis. With Quantisal collection tubes stored at room temperature (plastic separator in the extraction buffer) significant loss of THC was recorded; after 3 days THC concentration was reduced by almost 30 % and after 14 days, 60 % of the drug was lost. The Panel agreed that storage at room temperature should be avoided prior to the analysis of drugs for evidential testing purposes.

When stored under fluorescent lighting, the loss of THC was >50 % however, the presence of the pad (absorbent material) reduced the loss. When kept in the dark, the loss of THC at room temperature was approximately 20 % over 14 days whether the pad was present or not. Thus if fluorescent light and plastic tubes/surfaces are avoided (e.g. plastic separator is not allowed to remain in the sample) along with refrigeration of the specimen, or storage in the dark, the Quantisal™ collection system showed THC extraction >80 % and loss due to degradation <20 % over 2 weeks (Moore et al., 2006). Lee et al (Lee et al., 2012), also noted the importance of the Quantisal stabilization buffer, sample storage at 4 °C and analysis within 4 weeks, to maximize THC result accuracy.
Studies conducted with other commercial OF sampling systems have yielded the following results:

- With the Intercept® OF collection system, 13%, 45%, and 39% loss of THC in fortified OF has been reported after 2 weeks at −20 °C, 4 °C, and 21 °C, respectively; after 6 weeks, 21%, 87%, and 86% THC losses occurred (Crouch, 2005);
- With the StatSure Saliva Sampler™ authentic OF samples were re-analysed after room temperature (RT) storage for 1 week, and 4 °C for 1 and 4 weeks, and −20 °C for 4 and 24 weeks and THC concentrations were within 80 – 120% of the baseline for all storage conditions (Anizan et al., 2015);
- With the Oral-Eze sampler authentic OF samples were re-analysed at RT or after refrigerated storage (for 1 and 4 weeks) and THC concentrations were stable (94 – 100%) of baseline measurements (Anizan et al., 2015);
- With the StatSure Saliva Sampler™ THC was totally recovered from the collector pad after storage for 24 h at room temperature or 7 days at 4°C (Wille et al., 2013a).
- With the Quantisal collector a loss of 15 – 25% THC was observed whereas recovery from the Certus® device (Concateno, Alere Toxicology) was irreproducible (Wille et al., 2013a).

Buffer volumes for THC OF collection systems differ. The most diluted OF: buffer solution (Quantisal) had the lowest cannabinoid stability, whereas the least diluted OF: buffer solution (StatSure) exhibited the greatest stability. Less buffer however, results in lower total sample volume (Anizan et al., 2015); which may be problematic in circumstances where multiple drug confirmation assays on the same specimen are required, such as for drug driving offences. It is also currently the case that drug test results may differ for authentic and fortified OF. Test outcomes depend on the OF collection method, buffer composition and storage conditions; all may affect THC stability and therefore interpretation of the test result. A thorough investigation is advocated to determine which commercially available THC OF collection system would best suite the British drug-driving context.

4.8.2 Cocaine
The hydrolysis of cocaine and 6-MAM leading to the formation of BZE and morphine, respectively, was observed, in a stability study as follows (Ventura et al, 2007):

- After 3 and 7 days of storage at 37 °C (80% and 99.7% hydrolysis for cocaine and 37% and 82% for 6-MAM);
• After 7 days at 4 °C (23 % degradation for cocaine);
• After 1 and 2 month of storage at 4 °C for 6-MAM (15 % and 23 % degradation) and cocaine (82 %, and 93 %).

The addition of citrate buffer (Cone and Menchen, 1988), specifically at pH 4 and 0.1 % sodium azide to OF was found to prevent the degradation of cocaine for up to 7 days of storage at 25 °C and 37 °C and for up to 2 months of storage at 4 °C and -20 °C (Ventura et al., 2009). At -20 °C the percentage change after 2 months of storage, although statistically significant, was 5 % for cocaine. The Panel noted the importance of storage at or below 4 °C for OF samples thought to contain cocaine or 6-MAM.

4.8.3 Ketamine
See section on stability in blood

4.8.4 LSD
See section on stability in blood

4.8.5 Opiates and Opioids
The addition of citrate buffer (Cone and Menchen, 1988) at pH 4 and 0.1 % sodium azide was found to prevent the degradation of 6-MAM for up to 7 days of storage at 25 °C and 37 °C, and for up to 2 months of storage at 4 °C and -20 °C (Ventura et al., 2009). At -20 °C the percentage loss of 6-MAM after 2 months of storage was statistically significant at 10 %. The Panel noted the importance of storage at or below 4 °C for OF samples thought to contain 6-MAM. The Panel felt it was important to record that OF thought to contain 6-MAM should be buffered and stabilized to maintain sample integrity for the confirmatory analysis of illicit heroin. Further work is needed to explore if sodium azide impacts on the stability of other illicit drugs of interest.

4.8.6 Amphetamines
See section on stability in blood

4.8.7 Benzodiazepines
Samyn et al (Samyn et al., 2002a) found the stability of flunitrazepam was poor at 4 °C and the parent drug could only be detected when analysis was performed within 12 – 24 h after collection of the OF samples or when 2 % NaF was added to the collection tubes. In a more recent study, the stability of benzodiazepines using the Quantisal OF collection device and proprietary buffer was found to be poor for clonazepam (44 – 55 % recovery after 30 days at 4 °C) and flunitrazepam (48 – 50 % recovery after 30 days at 4 °C). It was recommended that OF samples thought to contain benzodiazepines should be stored at -20 °C if immediate analysis was not possible (Jang et al., 2013).
<table>
<thead>
<tr>
<th>Drug</th>
<th>Recovery Drug Collection Device/sampling system</th>
<th>Buffer/Preservative</th>
<th>Residence</th>
<th>Stability/Degradation</th>
<th>Contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC</td>
<td>Good for THC-COOH, THC was scarcely recovered with some systems (Langel et al., 2008)</td>
<td>Fortified OF more stable than authentic OF depending on composition buffer (Anizan et al., 2015)</td>
<td>Sample storage at 4 °C and analysis within 4 weeks to maximize THC result (Lee et al., 2012)</td>
<td>Loss THC under fluorescent lighting &gt;50% (Moore et al., 2006). Absorbs onto plastic tubes, rubber closures (Christophersen, 1986)</td>
<td>THC found in naso-oral cavities following smoking</td>
</tr>
<tr>
<td>Cocaine BZE</td>
<td>Reduced conc with stimulated OF collection (Kato et al., 1993)</td>
<td>Citrate buffer and sodium azide stops degradation cocaine (Cone and Menchen, 1988) (Ventura et al., 2009)</td>
<td>Storage below 4 °C preferable</td>
<td>At ambient temp 26 % to 41 % of cocaine converted to BZE (Langel et al., 2008)</td>
<td>Cocaine found in naso-oral cavities following smoking</td>
</tr>
<tr>
<td>Ketamine</td>
<td>Not known</td>
<td></td>
<td></td>
<td>Stable 4°C for 2 days or -20°C for 3 month (Hijazi et al., 2001)</td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>Not known</td>
<td>LSD lost if stored transparent tubes (Klette et al., 2002)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphetamine Methamphetamine MDMA</td>
<td>Easily detected with POCT tests (Engblom et al., 2007); cross-reactivity may</td>
<td>Increase in pH (during salivation) results in a reduction concentration of amphetamine in OF (Navarro et al., 2001)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Amphetamine MDMA*
<table>
<thead>
<tr>
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<th>Contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-MAM Morphine Methadone</td>
<td></td>
<td>Citrate buffer (Cone and Menchen, 1988), sodium azide stops degradation</td>
<td>High prevalence 6-MAM in OF.</td>
<td></td>
<td>Opiate false positives with poppy seed ingestion (Concheiro et al., 2015)</td>
</tr>
<tr>
<td>Methadone</td>
<td></td>
<td>Methadone predominates over EDDP in OF</td>
<td>High OF pH causes significant decrease in methadone conc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzodiazepines Diazepam</td>
<td>Nordiazepam and 7-amino clonazepam predominate in OF (Melanson et al., 2016).</td>
<td>Flunitrazepam needs 2 % NaF for stability (Samyn et al., 2002a)</td>
<td>POCT devices often fail to detect benzodiazepine (Jones et al., 2014)</td>
<td>Poor recovery clonazepam and flunitrazepam at 4 °C (Jang et al., 2013).</td>
<td></td>
</tr>
</tbody>
</table>

*study conducted in urine*
Based on what is known, OF samples should be refrigerated (4 °C) as quickly as possible after collection and transported in a controlled temperature to avoid bacterial contamination and degradation of drugs (Table 26). The addition of sodium azide inhibits bacterial growth and preserves samples but may affect some immunoassay type tests (Nunes et al., 2015). The Panel recommend that standard collection procedures are agreed and note that these exist and have been published by the Standards Forum in Australia for the quantitation of drugs in OF (Drummer, 2006).

There is growing evidence that OF samples should be analysed as soon as possible after collection, and stored at -20 °C if immediate analysis is not possible (Lund et al., 2011), but for no longer than 4 weeks (see summary Table 26).

The Panel agreed that a defined minimum specification for OF collection tubes for quality assurance purposes would be helpful. Random sampling of collection kits and tubes would help confirm buffer and preservative concentrations within agreed published limits. The Panel also felt that a shelf life for OF collection tubes should be published and monitored.

The Panel recommended that a minimum of 2mL of OF would be required for evidential drug testing purposes and that OF sample collection tubes should be specified (minimum standard) for this purpose as detailed below.

The Panel noted that in order to introduce OF for evidential testing the Department for Transport, Home Office, police authority and the Forensic Service Provider (FSPs) would need to agree written protocols for the collection, storage and dispatch of OF to the laboratory. Notification of the time of sample collection would remain, as for blood, a necessity. Recording testimony about self-reported drug use/consumption would also be helpful.

### 4.9 Specification of Collection Kit for OF

The specification of the OF sample collection kit would match that required for blood (as below). Specifications have been reported for OF testing in the workplace and are a useful reference (Cooper et al., 2011).

- Tamper proof, sealed tube
- Laboratory-based evaluation process
- Quality control (testing before use)
- Date of manufacture/batch number
New guidelines for police officers with regard to the collection of OF for evidential tests would be needed that included advice to the defendant with regard to the accredited laboratories that can analyse his/her samples.

4.9.1 Specification for the OF collection tube
The Panel agreed that there should be a minimum specification for OF sample collection tubes with the following recommendations:

- Sealed glass tube with a neoprene lined cap e.g., Nunc Cryo-Tubes™ (Thermo Scientific) (Lee et al., 2012)
- Indicator for amount of OF volume obtained (ideally 2 - 4 mL) within ± 10 %
- Final concentration of sodium azide should be a minimum 0.1 % w/v after the addition of OF; the content should be managed with published details of batch number, shelf life and quality control;
- Final concentration of citrate buffer should achieve pH 4 and confirmation of citrate buffer content should be managed with published details of batch number, shelf life and quality control.

4.10 Susceptibility of OF to Contamination or Adulteration
Research has been carried out to look at factors that may affect the detection of drugs in OF. Reichardt et al (Reichardt et al., 2013) investigated the effect that food or beverages may exert on OF screening tests. Results showed that intermittent presumptive positive results for amphetamine, methadone, opiates and cocaine could be obtained following the consumption of coffee, Coca Cola®, fruit juice, oranges, spicy food and toothpaste using the Orasure OF sampling system (Orasure Technologies Inc, 2016). Following the consumption of vinegar, presumptive positives were observed using the Orasure system for up to 30 minute post-exposure; vinegar can affect immunoassay screening for an extended period following its consumption (Reichardt et al., 2013).

However the use of commercial adulterants or other products capable of acting as adulterants, such as Clear Choice®, Fizzy Flush™, Spit and Clean®/™ mouth wash and Cool Mint Listerine® had no substantial effect on the Oratect® POCT OF drug screening device test 30 min after exposure (Wong et al., 2005).

When drugs are consumed by oral, intra-nasal, smoking or inhaling (insufflation) routes of administration, or taken sublingually e.g., buprenorphine (Melanson, 2009), the time of sample collection is relevant. Drugs can accumulate in the oral cavity and produce elevated concentrations in OF for several hours after ingestion by drug users (Cone, 1993, Niedbala et al., 2001), and this may cause problems with interpretation (Allen, 2011).
zero tolerance approach ruling out accidental exposure is therefore recommended and why countries such as Spain and Australia have taken this approach.

In a study to investigate possible false positives for opiate drug tests due to consumption of poppy seeds, 17 healthy adults were administered two 45 g raw poppy seed doses (15.7 mg morphine, 3.1 mg codeine). All OF specimens (n = 459) were screened using the Dräger DrugTest 5000, and confirmed with OF collected using the Oral-Eze® sampling system before analysis by LC-MS/MS. All OF specimens screened positive 0.5 hours after dosing and remained positive for 0.5 – 13 hours at the Dräger 20 µg/L morphine cut-off. After two doses, the last morphine positive OF result was 0.5 hour with a 95 µg/L cut-off, recently recommended by the DRUID project and 1 hour using the SAMHSA 40 µg/L cut-off (DRUID, 2012, SAMHSA, 2015). It was concluded that positive OF morphine drug test results are possible 0.5 – 1 hours after ingestion of 15.7 mg of morphine in raw poppy seeds, depending on the cut-off employed (Concheiro et al., 2015).

4.11 OF - SUMMARY
OF is readily accessible and collectible. It has become of interest as a viable matrix material for drug testing because no medical personnel are needed for sampling. This matrix is especially applicable for preliminary drug testing (screening), where immediate results are required. Indeed, the development of sensitive methods for drug testing in OF over the last few years has led a number of jurisdictions around the world to adopt OF as a screening tool for the detection of illicit drugs and psychoactive medicines in those suspected of drug-driving offences (Lillsunde, 2008).

OF as a matrix for confirmation of driving offences is currently being debated. Discussion points include a shorter detection window (compared with urine), the effects of pH variation on the appearance of drugs in OF and the potential for buccal cavity contamination. At present OF screening is predominantly conducted using POCT immunoassay drug test kits and a small volume of OF is made available at the collection site. Detection of more than one drug and in some cases at a lower concentration than in blood may become a significant challenge if only small quantities of OF were available for analysis. It is likely that significantly more OF would be needed to potentially quantify 17 different compounds for evidential purposes. Estimates suggest that 2-4 mL would be required by FSPs.

Scientists have concluded that the wide range of OF: blood ratios will not allow reliable estimation of blood concentrations from OF concentrations, making the use of OF for evidential testing using the per se (threshold) approach difficult (Wille et al., 2009,
Drummer, 2006, Lacey et al., 2009). However, if confirmatory testing was to be established based on a cut-off at the LLOQ then OF has potential.

There would be practical limitations to overcome in order to use OF for evidential testing. POCT OF drug screening processes using immunoassay test kits usually involves a small volume of OF and do not give definitive concentrations for specific compounds. However, a two stage procedure using an initial screen with DrugWipe® (Securetec, 2016) and confirmation using a different POCT, the OF Cozart® Rapiscan (Alere™, 2016a) has been successfully used in the State of Victoria, Australia. If positive half of the OF collected in the second test is sent to a laboratory for evidential verification. Should such an approach be envisaged in the UK and given the variability in performance of the commercial OF POCT devices on the market, the Panel recommend that criteria in terms of sensitivity, specificity and accuracy are established for the OF POCT device(s) to be employed. Drug test accuracy of ≥ 95%, sensitivity of ≥ 90 % and, specificity of ≥ 90 % are the required performance criteria in the State of Victoria. Minimum standards were also established by the European integrated project DRUID and was set at 80 % for each parameter; the Dräger DrugTest (DCD) 5000® type-approved by the Home Office fulfilled the DRUID criteria for all drug classes included in the section 5A offence.

Evidential testing to quantify up to 17 compounds would likely require significantly more OF than is currently used in POCT roadside screening tests; estimates by FSPs suggest 2 – 4 mL would be required. The collection of OF would therefore need to involve a commercial OF sampling system. OF commercial sampling systems are many and varied with important differences in performance for evidential drug testing. The proprietary buffers used are not uniform and differing volumes of OF and buffer solutions are in place, which affect the reporting quantitative results. If OF is to be collected using a commercial sampling system for evidential purposes then the Panel recommend that recovery of the OF from the collection sampler as well as the recovery of analytes of interest and the overall reliability of the system would need to be well characterised and accredited for use at the road-side or in the police station.

The Panel noted that drug recovery data would need to be used in conjunction with collection volume imprecision data (i.e. whether the OF collected was above, below or had achieved the minimum volume required) and uncertainty of measurement to provide an estimation of drug concentration in neat, authentic OF. The sample collection kit and the OF collection tube equipment would need to be independently assessed to a specification (minimum standard) for so that it meets the above standards. This might be part of the FSP accreditation process (see section 11).
Based on what is known, the Panel recommends that OF samples should be refrigerated (3 – 5 °C) as quickly as possible after collection and transported to the laboratory as quickly as possible in a controlled temperature, away from fluorescent and natural light to avoid bacterial contamination and degradation of drugs. It is also recommended that OF samples be stored in glass tubes and should be frozen (ideally at -20 °C), if not available for immediate analysis.

OF as a possible matrix for confirmatory testing, may have other limitations. Potential confounders include the effects of pH variation on the appearance of drugs in OF, and the potential for buccal cavity contamination. Dry mouth syndrome and/or xerostomia, which can be brought about by drug use (cannabis, cocaine and the opiates) may inhibit an individual’s ability to produce a viable sample.

The Panel noted that a number of considerations with regard to the legislation relating to the section 5A offence would also need to be discussed:

1) That changes to primary legislation would need to be sought to allow for OF to be added as a matrix for the section 5A drug driving offence;
2) That where OF were to be set in regulations these would need to be equivalent as far as possible to the existing limits set in the regulations for whole blood;
3) That Parliament would need to decide whether OF was to be an alternative to blood at the police officer’s discretion or an alternative, only to be required when there is an acceptable objection to blood;
4) That a discussion could usefully take place to consider whether OF should also be allowed as an evidential matrix for section 4 impairment cases, in addition to urine and blood;
5) That the pre-requisite in Section 7 of the legislation providing that a blood or urine specimen cannot be required for drug purposes unless a Doctor or Healthcare Professional (HCP) has determined that the person has a ‘condition which might be due to some drug’ or there has been a positive preliminary drug test is extended to the provision of evidential OF.

To conclude, there is a stronger argument for the use of OF as an evidential matrix when using laboratory based cut-offs (LLOQ) such as those suggested by DRUID (DRUID, 2012), as the concentration above which an offence would occur. This approach would be in line with a zero tolerance approach, rather than a road safety risk based approach. With regards to the drug limits in the section 5A regulations, OF limits could not be identified for the controlled medicinal drugs where a risk-based approach underpins the concentrations in whole blood. Nevertheless, such a division may be problematic for
police in deciding what type of specimen to require where, as in most instances, they have little or no foreknowledge of the drugs in question.
5 SWEAT

The secretion of sweat occurs by at least two distinct pathways; by passive diffusion of fluids, including water, through the skin, and by glandular secretion, with the process of sweating. The rate of fluid loss depends on the extension of the body surface, ambient temperature, body temperature, and the relative humidity of the environment (UNDOC, 2014). Kidwell et al (Kidwell et al., 2003) noted that a number of drugs have been detected in sweat at concentrations directly proportional to those found in plasma and reported the potential of sweat as an effective matrix for drug testing.

Sweat patch testing has been used to ensure abstention and compliance in drug treatment programmes and in the United States extensively as a deterrent to drug use in the criminal justice system (Preston et al., 1999, Baer and Booher, 1994). Samples of sweat has also been used for continuous monitoring of drug use over 1 – 14 days (Dolan et al., 2004), in treatment, and employment settings and most recently to screen suspected drug-drivers at the roadside (Gentili et al., 2016).

5.1 RESIDENCE AND DETECTION TIME OF DRUGS IN SWEAT

It is agreed that parent drugs are generally excreted in sweat at higher concentrations than their metabolites (Cone et al., 1994b). This is because of their greater lipophilicity and the fact that basic compounds tend to accumulate in sweat due to ion-trapping in the more acidic conditions (Huestis et al., 1999).

5.2 COLLECTION METHODS AND DEVICES USED FOR SWEAT COLLECTION

The PharmChek® sweat patch is a well-known sample collection tool that has been available for the detection of cocaine, opiates and methamphetamine since 1995 and, for cannabis and phencyclidine since 1996 (Pharmchem Inc, 2016). The sweat patch is attached to the upper arm or torso and they are worn for a minimum of 24 h and can collect sample for between 7 and 10 days.

Other devices such as the Hand-held Fast Patch and the Torso Fast Patch have been described (Huestis et al., 1999). More recently, the DrugWipe® (Securetec Detektions-Systeme AG) sweat screening device has been introduced. It is an immunochemical strip test that collects sweat from the forehead to detect drugs of abuse.

Various drugs have been detected in sweat including buprenorphine, methadone, cocaine, opiates, amphetamine and nicotine metabolites (Concheiro et al., 2011a, De Giovanni and Fucci, 2013). Specific examples are given below:
5.2.1 Cannabis
PharmChek® sweat patches have been used to evaluate THC excretion in daily cannabis users. Sweat patches worn the first week had THC above the SAMHSA (United States Substance Abuse Mental Health Services Administration) proposed cut-off concentration for federal workplace testing of 1 ng THC/patch (mean ± SE, 3.85 ± 0.86 ng THC/patch) (Pharmchem Inc, 2016). In 7 subjects who were administered oral doses (14.8 mg THC/day for five consecutive days), no daily or weekly patches had THC >0.4 ng THC/Patch; concurrent plasma THC concentrations were <6.1 µg/L (Huestis et al., 2008).

5.2.2 Cocaine and BZE
The excretion of cocaine and its metabolites into sweat have been clearly demonstrated and doses as small as 1 to 5 mg have produced detectable concentrations (Kidwell et al., 2003). Kacinko et al (Kacinko et al., 2005) found that sweat patches were useful for monitoring cocaine exposure and following administration of three doses of cocaine hydrochloride (75mg/70kg) subcutaneously, to nine subjects for a week, and three higher doses (150mg/70kg) 3 weeks later cocaine was detected within an hour and BZE within 4 – 8 h. No statistically significant difference was found for sweat test results between low and high dosing at 15 h. It was concluded that monitoring cocaine use via sweat patches was effective if worn for 7 days against a confirmation cut-off of 25 ng/patch as suggested in the SAMHSA guidelines (SAMHSA, 2015).

In sweat BZE appears slow to emerge; Cone et al found that BZE was only detected at high doses (25 mg) (Cone et al., 1994b), whilst following uncontrolled drug consumption cocaine was detected within 2 h, but neither BZE nor EME appeared until 24 h after self-reported use (Liberty et al., 2004).

Cocaine administered by different routes of administration; smoked (42 mg), intravenous (25 mg) and intranasally (32 mg) appeared in the sweat patch within 24 h, with little additional drug detected over the next 48 h. A similar finding was made by Burns & Baselt (Burns and Baselt, 1995), who gave 18 subjects either 50 mg or 126 mg of cocaine intranasally. A clear indication of a dose-response relationship was observed although the degree of intra- and inter-subject variability was deemed too great for the use of sweat patches as a means to achieve meaningful quantitative interpretation.

5.2.3 Ketamine
Ketamine has not been extensively studied in sweat. Demoranville et al reported the use of ion mobility spectrometry (IMS) for the determination of ketamine in simulated sweat (5 ng deposits) as a presumptive test (Demoranville and Verkouteren, 2013).
5.2.4 Opiates and opioids

Following dosing with codeine sulphate, codeine was the only analyte identified in hourly (12.6 %) and weekly PharmChek® sweat patches (83.3 %). Hourly patches were applied for 1 to 15 h (n = 775) and weekly patches for 7 days (n = 118). Weekly patch concentrations were 38.6 ± 59.9 ng/patch (range, 0 – 225 ng/patch) for low and 34.1 ± 32.7 ng/patch (range 0 – 96 ng/patch) for high codeine doses. Codeine could still be detected in some patches 1 week after dosing; mean 4.6 ± 5.3 ng/patch, range, 0 – 17 ng/patch after low and mean 7.7 ± 7.1 ng/patch, range 0 – 21 ng/patch after high doses. Hourly sweat patch tests were considered to be less effective than those that collected sweat that had accumulated over a week. Only 2.6 % of hourly compared to 45.5 % (high-dose) weekly patches containing codeine at the proposed SAMHSA cut-off (Schwilke et al., 2006).

5.2.5 Amphetamines

It has been known for some time that amphetamine and methamphetamine are excreted into sweat (Vree et al., 1972); more than thirty years ago Ishiyama (Ishiyama et al., 1979) recorded that methamphetamine given orally in doses of 10 mg is excreted in the sweat at a constant rate (1.4 μg/mL). Barnes et al studied the excretion patterns of amphetamine and methamphetamine into human sweat following four 10 mg oral doses of sustained-release S-methamphetamine and found that methamphetamine was measurable in sweat within 2 h of dosing; 92.9 % of weekly PharmChek® sweat patches were positive, with a median of 63.0 (range 16.8 – 175) ng/patch; amphetamine concentrations were 15.5 (range 6.5 – 40.5) ng/patch. Patches applied 2 weeks after dosing had no measurable methamphetamine concentration (Barnes et al., 2008).

When MDMA was given to healthy volunteers (n = 15) with histories of MDMA use, concentrations up to 3007 ng/patch MDMA were observed. PharmChek® patches (up to 5 at one time) were applied to the back or abdomen and worn for intervals ranging from 2.5 h to 1 week. Four types of patches were used including washout, short-term (worn for 12 h or less), daily and weekly patches. Of 559 sweat patches collected during and after controlled oral MDMA administration, 370 contained either MDMA or MDA, with no patches positive for HMMA or HMA. At an LLOQ of 2.5 ng/patch, 64.4 % of patches were positive for MDMA, whereas 38.8 % were positive at the proposed SAMHSA cut-off of 25 ng/patch. For MDA, 31.1 % were positive at the 2.5 ng/patch cut-off and 10.4 % at 25 ng/patch (Barnes et al., 2009).

In two healthy volunteers familiar with the effects of MDMA, given 100 mg of the drug as a single dose, consumption of MDMA was detected in sweat following armpit swabbing at 2 h and for up to 12 h after administration (Pacifici et al., 2001).
5.2.6 Benzodiazepines
Following oral administration of a single dose of diazepam sweat was collected by means of the Sudormed™ sweat patch. Patches were removed at specified times over one week and drug content was determined by GC-MS. It was found that irrespective of the time of collection, diazepam and nordiazepam were present, but oxazepam was never detected. Drugs were detectable from 2 to 4 h after administration. Concentrations were in the range from 0.1 to 6.0 ng/patch for both drugs (Kintz et al., 1996). More recently, the Drugwipe® sweat test has been reported to detect various benzodiazepines at a LOD of 5 µg/L (Aberl and VanDine, 2005).

5.2.7 Roadside drug testing using sweat
In Belgium from 1999 until 2000 newly trained police officers evaluated drivers using OF and sweat POCT tests at special enforcement roadblocks. Drivers who failed a sobriety test were screened by using the Drugwipe® device (Securetec, Germany) as a sweat test (by wiping across the forehead) or as an OF test (by wiping the tongue). The reliability of the Drugwipe® was assessed by comparing its roadside results with confirmatory tests in plasma using GC-MS. The accuracy of Drugwipe® using sweat was 75 %, 68 % and 95 % for opiates, cocaine, and amphetamines and deemed reliable for amphetamine drug testing, including MDMA (Samyn et al., 2002b, Samyn and van Haeren, 2000).

The Drugwipe® POCT device has been widely in Germany as a routine roadside sweat (or OF) screening test for those apprehended for driving under the influence of drugs (DUID). Based on 1763 cases, a statistical evaluation by traffic police in Germany shows that more than 97 % of all positive Drugwipe® sweat tests were confirmed with positive blood results (Aberl and VanDine, 2005). The Drugwipe® sweat screening device has also been used successfully for roadside drug tests in Italy. During 2015 in Northern Italy, the Italian police tested drivers stopped for suspected drug-driving. GC-MS analysis showed 44 out of 66 sweat tests were positive for one substance; MDMA (10 ng/pad), cocaine (10.1–600.9 ng/pad) or THC (0.5–14.1 ng/pad); 16 samples were positive to two or more substances. There were 4 MDMA, one cocaine and 7 THC false positive results (Gentili et al., 2016).

Cut-off concentrations (µg/L) for sweat testing, according to the manufacturers specification, for the Drugwipe® POCT screening device and of relevance to the section 5A legislation, are reported below (UNDOC, 2014):
### Table 27  
Cut-off concentrations (µg/L) for sweat testing, for the Drugwipe® POCT screening device

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Specific compound</th>
<th>Cut-off concentration (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannabis</td>
<td>THC</td>
<td>30</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Cocaine</td>
<td>50</td>
</tr>
<tr>
<td>Opioids</td>
<td>Heroin</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Morphine</td>
<td>20</td>
</tr>
<tr>
<td>Amphetamines</td>
<td>d-amphetamine</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>MDMA</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>d-methamphetamine</td>
<td>100</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>Flunitrazepam</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Temazepam</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Diazepam</td>
<td>10</td>
</tr>
</tbody>
</table>

#### 5.3 Susceptibility of sweat to contamination or adulteration

Problems reported for the use of sweat patches for monitoring cocaine abuse were noted by Kidwell and Smith (Kidwell and Smith, 2001) to include accidental contamination. Positive sweat patches were found in drug-free volunteers who had cocaine placed on their skin surface and washed with normal hygiene procedures, in addition to recommended cleaning procedures, prior to application of sweat patches. External contamination may also be an issue with other drugs handles before use.

#### 5.4 Sweat testing - Summary

There is evidence from Italy, Belgium and Germany that sweat testing can be successfully used at the roadside as a preliminary tool to investigate road traffic offences. The use of a sweat POCT screening device has been employed successfully to test those apprehended at the roadside and thought to be under the influence of drugs. Sweat testing per se however has yet to be shown as applicable for confirmatory drug-driving tests. Consideration would need to be given to the issue of external contamination and how this can be negated as part of the sample collection procedure.
6 HAIR

Testing human scalp hair for the presence of drugs may offer a further option for confirmatory drug testing. This technique has the potential for examination over a much longer time-scale (1 – 3 month) than is possible with blood, urine and OF. Scalp hair analysis may offer a more dignified collection procedure than that for OF, urine or blood, with minimal risk of infection and little opportunity for evasion or adulteration of the sample (Brewer, 1990, Brewer, 1993).

6.1 RESIDENCE AND DETECTION TIME OF DRUGS IN HAIR

The analytical procedure for the detection of drugs in hair requires highly sophisticated analytical equipment, the analysis is not straightforward and not undertaken routinely by forensic laboratories. There are several sources of error and misinterpretations, which have been documented (Pragst and Balikova, 2006); in some cases due to the presence of drugs in very low concentrations (10 pg – 10 ng/mg hair), in other circumstances due to external contamination or passive exposure (Cassani and Spiehler, 1993).

6.2 COLLECTION METHODS AND SETTINGS FOR HAIR SAMPLE COLLECTION

A practical difficulty with this matrix is the dependence upon the nature of the hair style. Drug screening usually requires a lock of hair ideally 1 to 3 cm in length, equivalent to the thickness of a small lead pencil (30 to 50 mg) to be taken from the crown of the head; although newer developments suggest analysis is possible using a single strand of hair (Kintz, 2013). Correlation of sample time with drug use requires identification and alignment of the cut ends of hair (Strang et al., 1990, Marsh and Evans, 1994, Marsh et al., 1995) and hence sampling of hair growing from a closely cropped head is more difficult and is likely to contain lower concentrations after the same dose of drug as hair that has not been cut over a long period of time with variations in the concentrations of up to 20% (Sachs, 1995).

Hair grows at a rate of about 1 cm/month and is thought to contain the amount of drug corresponding to that ingested over this period (Sachs, 1995). Drug concentrations in hair samples are generally highest in the root (scalp) and lowest at the distal end. The variability of hair growth rate both within and between individuals reduces the accuracy of linking drug use to specific periods of time (Nakahara et al., 1992a, Nakahara et al., 1992b, Nakahara et al., 1991, Nakahara et al., 1990). Scalp hair from the posterior vertex region or crown is thought less susceptible to such variations in growth.
International guidelines exist for drug and alcohol testing in hair, such as the European Workplace Guidelines (Salomone et al., 2016, Agius and Kintz, 2010), which were approved in 2011 by the European Cooperation for Accreditation (EA) as an advisory document. In Germany hair testing has been used as part of the re-granting of driving licences under the Medical and Psychological Assessment (MPA) scheme (Dufaux et al., 2012, Agius and Nadulski, 2014, Agius et al., 2013). The evidential cut-offs levels are as follows (Table 28):

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cut-off in Hair (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC</td>
<td>0.02</td>
</tr>
<tr>
<td>Cocaine</td>
<td>0.1</td>
</tr>
<tr>
<td>Methadone and EDDP</td>
<td>0.1</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>0.1</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Most workplace guides recommend that employees should be notified one month before sample collection is due to take place to allow the hair to grow out of the scalp and be sufficiently long to facilitate hair collection (Bush, 2008, Agius and Kintz, 2010). This requirement could obviously not be accommodated for sampling immediately following a drug-driving offence.

6.3 STABILITY OF THE DRUGS IN HAIR

The extent of the axial diffusion of drugs along the hair shaft may vary widely between individuals, particularly for cocaine and methadone (Nakahara et al., 1995) and hair concentrations of substances such as the amphetamines may decrease or decompose over time (Balabanova and Albert, 1994). A further source of inconsistency is the incorporation rate (ICR) of drugs into hair which varies markedly from high concentrations in natural hair (relative to the melanin content) to very low concentrations in treated (bleached) hair. Basic drugs (including opiates, amphetamine and cocaine) are more easily incorporated (Nakahara et al., 1995) than acidic drugs (Nakahara and Kikura, 1996). It is thought to take about a week for residence to occur in hair following drug use. Hence to
confirm recent drug use a time lag of at least 5 – 7 days is required before sampling. For forensic testing a time lag of 3 – 4 weeks is often suggested. Thus the link between impairment and driving performance would not be easily made.

6.4 SUSCEPTIBILITY OF HAIR TO CONTAMINATION OR ADULTERATION

Passive contamination of the external surface of the hair from the environment (Wang and Cone, 1995) is a well-documented issue and presents interpretational problems (Blank and Kidwell, 1995, Blank and Kidwell, 1993) and a risk of false positives (Romano et al., 2001). Moreover, some hair types, such as thick black hair, have been reported to be more resistant to decontamination procedures than others (Blank and Kidwell, 1993, Blank and Kidwell, 1995). Common hair treatments such as basic perms may increase drug absorption into hair.

Some specific details about the compounds included in the drug-driving legislation are included below:

6.4.1 Cannabis

THC the primary active constituent of cannabis, being highly protein bound in plasma and some metabolites (THC COOH) may be repelled by the hair matrix due to a lack of affinity for melanin. THC appears to be particularly difficult to determine in hair (Nakahara and Kikura, 1994, Cirimele et al., 1995, Kintz et al., 1995, Staub, 1999), although methodology has been described by (Khajuria and Nayak, 2014). Using GC-MS Khajuria et al achieved a LOD 0.1 ng/mg of THC and detected a mean THC 0.95 ng/mg (range 0.16 to 2.3 ng/mg) with THC detectable for up to 3 months after the last drug intake. Issues have also been raised concerning efforts to distinguish passive exposure to cannabis from active ingestion (Thieme et al., 2014, Uhl and Sachs, 2004). Smoke deposits from cannabis preparations contain THC and diffusion into hair may lead to an incorporation that cannot be distinguished from internal sources i.e., consumption (Thorspecken et al., 2004).

Data on the incorporation of THC and THC-COOH into hair suggests THC is found in the hair of individuals who handle cannabis material, whilst THC may not be incorporated into human hair in relevant amounts after systemic uptake. Cannabinoids can be present in the hair of non-consuming individuals because of transfer through handling cannabis from their hands into sebum and sweat, or through passive exposure (Moosmann et al., 2015).

6.4.2 Cocaine and BZE

Understanding the mechanisms and timeline of a drugs disappearance from hair is critical for evidential testing. After the discontinuation of drug use the median elimination half-
life of cocaine in hair was 1.5 month (95% CI 1.2 - 1.8) in females and in males (95% CI 1.1 - 1.8). The median half-life of BZE was also 1.5 month (95% CI 1.1 - 2) in females and males (95% CI 0.8 - 1.8), (Mann-Whitney U-test; P = 0.93 for cocaine, P = 0.99 for BZE). Cocaine and BZE has been detected in the hair of former drug users for several months after abstinence; approximately 3 – 4 months have to pass before hair testing becomes negative in the segment proximal to the scalp (Romano et al., 2001). BZE is produced by degradation of cocaine in the environment, and other metabolites may be detected because of contaminants in the hair of cocaine users (Romano et al., 2001). Past and current data show that cocaine is readily incorporated into hair from environmental exposure and not normally removed by common decontamination procedures (Kidwell and Smith, 2016, Kidwell et al., 2015).

6.4.3 Ketamine
Ketamine in its neutral form, strongly interacts with melanin, facilitating its incorporation into hair. Numerous chromatographic methods have been published for the detection of ketamine and its metabolite norketamine in hair (Leong et al., 2005, Parkin et al., 2008, Harun et al., 2010, Tabernero et al., 2009, Favretto et al., 2013). Salomone et al (Salomone et al., 2015) detected ketamine and norketamine in hair in 15 cases, with minimum concentrations of 0.11 ng/mg and 0.02 ng/mg respectively. Norketamine was detected in all samples. Passive exposure to ketamine detected in hair has been reported (Wu et al., 2008).

6.4.4 LSD
A dedicated LC-electrospray-MS/MS assay has been used to document the case of a fatality involving LSD in which a concentration of 0.66 pg/mg LSD in pubic hair was observed (Gaulier et al., 2012). Passive contamination of hair from LSD has not been reported.

6.4.5 Opiates and opioids
Segmental hair analysis has proved useful to identify methadone and other opioids in hair following fatal poisoning in drug addicts in Denmark. In hair, methadone was detected in 97 fatalities with concentrations ranging from 0.061 to 0.211 ng/mg. 6-MAM was detected in 30% of fatalities in the proximal hair sample, while only one blood sample was positive for 6-MAM, indicating previous recent use of heroin (Nielsen et al., 2015).

6.4.6 Amphetamines
The direct analysis of amphetamines in hair has been achieved with LC and fluorescence detection with LOD ranging from 0.25 to 0.75 ng/mg (Argente-Garcia et al., 2016), although external contamination through exposure to fumes or vapours has been
reported. From 52 potential cases of passive exposure examined, 38 (73 %) were positive for methamphetamine (>0.1 ng/mg) and amphetamine was detected in 34 of these cases (Bassindale, 2012).

6.4.7 Benzodiazepines
Xiang et al in a review, reported that benzodiazepines including clonazepam, diazepam, flunitrazepam, lorazepam, nordiazepam, oxazepam, temazepam, and triazolam were detectable in human hair after a single therapeutic dose. However concluded that the physicochemical properties of the drugs, hair colour, sample preparation, axial distribution and inter-subject could affect benzodiazepine hair concentration (Xiang et al., 2015).

6.5 Hair-Summary
Hair testing has been used in many European countries to confirm abstinence from illicit drugs in persons whose driving licences have been suspended for drug-impaired driving. For instance, in Italy hair testing has been used to verify current and past abstinence of cocaine in those reapplying for a suspended driver’s licence (Polla et al., 2009).

The Panel felt that this was an appropriate use of the matrix since hair testing provides a much longer window of detection (timeframe of use) than other matrices. Sample collection of hair for testing at the road-side would not seem practicable and the time-lag between drug deposition in hair and consumption would make the interpretation of hair tests for evidential testing rather complicated.

Hair analysis remains useful in establishing a history of past exposures to therapeutic or abused substances over a longer period of time than is usually possible with OF, blood or urine. Hair testing would seem a sensible candidate for confirming abstinence in high-risk drug-drivers or in those who are applying for re-licensing.
7 INNOVATIVE SAMPLES

The Panel sought information about the usefulness of alternative matrices and/or different sampling approaches that may in the future be an avenue for consideration with regards to evidential testing in the drug-driving context.

Dried blood spots or small liquid samples offer an alternative to blood taken by venepuncture and therefore may negate the need for clinical staff (forensic medical examiner, nurse, or paramedic) to attend to draw a sample from an arrested driver. Apart from the cost saving, if the sample can be taken by police staff then the time delay between the road traffic incident that necessitated the arrest and the sample being taken would be kept to a minimum. Drug in latent fingerprints and breath are entirely novel samples that would need a great deal of further work and development before they could be adopted. Nevertheless these matrices may have a role and their current development is considered briefly below.

7.1 DRIED BLOOD SPOTS (DBS)
The use of dried blood spots for the collection of samples for analysis is not new. The idea of using blood collected on a paper card made of cellulose can be ascribed to Ivar Bang who reported determining glucose from eluates of dried blood spots (DBS) in 1913 (Bang and Bergmann, 1913). More recently, the concept that capillary blood, obtained from pricking the heel or finger and blotted onto filter paper, could be used to screen for metabolic diseases in large populations of neonates was introduced in Scotland by Robert Guthrie in 1963 (Guthrie and Susi, 1963). The Center for Disease Control and Prevention in the United States of America lists a large number and variety of substances that have been measured using dried-blood spots and this list is by no means exhaustive (CDC, 2016b). A more comprehensive database has been compiled by a commercial company, Spot On Sciences Inc. Their Dried Blood Spot (DBS) Methodology Database has been created to allow easy access to published analytical methods based on DBS technology (Spot On Sciences, 2016a).

7.1.1 Residence and detection time of drugs in DBS
Some initial work was carried out by Hammond et al (Hammond et al., 1979) or the extraction and analysis of benzodiazepines in DBS but it has not been until more recently that the utility of DBS for drug and pharmaceutical analysis has been recognised (Li et al., 2012, Desai and Ravindra, 2013, Sharma et al., 2014). The greater uptake and availability of LC- and GC-MS has enabled the quantification of drugs in the small quantities of blood...
collected, 20 µL to 50µL, on the absorbent paper (Li and Tse, 2010, Keevil, 2011, Wilhelm et al., 2014). Head to head comparisons of samples collected by dried blood spots and venous blood sampling have demonstrated good agreement between the two sampling methods for forensically important compounds, for example paracetamol (Taylor et al., 2013), carbamazepine (Kong et al., 2014), γ-hydroxybutyrate (Sadones et al., 2015), and ephedrine and methyl-ephedrine (Kojima et al., 2016).

7.1.2 Collection methods and devices used for DBS sample collection
The procedure for taking samples is simple. Using a spring-loaded lancet a finger is pricked and the blood that bleeds from the puncture wound is collected onto specially treated absorbent paper. More than one spot can be collected from the bleeding wound. The blood on the paper is then allowed to dry and then placed in a plastic bag for storage and onward shipping to the laboratory. There is generally no requirement for refrigeration. DBS specimens can be shipped by mail, or other carrier, with no reasonable expectations of occupational exposure to blood or other potentially infectious dried-blood material (CDC, 2016a). The Royal Mail guidance on the shipping of potentially infectious samples specifically exempts dried blood spots from their Dangerous Goods Regulations (DGR);

“Dried blood spots, collected by applying a drop of blood onto absorbent material, or faecal occult blood screening tests and blood or blood components which have been collected for the purposes of transfusion or for the preparation of blood products to be used for transfusion or transplantation and any tissues or organs intended for use in transplantation are not subject to the DGR” (Royal Mail, 2016).

There have been several proposals for the evaluating and defining sample preparation procedures for DBS from individual laboratories (Liu et al., 2010) and from organisations such as the European Bioanalysis Forum (Timmerman et al., 2013) and regulators including the USA’s Food and Drug Administration (FDA) (Smeraglia et al., 2014) and these recommendations have gone through several iterations (Timmerman et al., 2014). Sources of variability identified include within and between lot variations in the paper use, differences between manufacturers, inconsistencies in the printing and cutting-out of the “spots”, humidity of the paper, the volume of blood collected, absorption time for the blood, sampling of the blood, and handling and storage of the paper.

Variations in the haematocrit of the blood is one of the major factors relating to the sample which complicates DBS sampling for analysis (de Vries et al., 2013). Variability in blood haematocrit needs even to be considered when preparing standards and quality control samples (Koster et al., 2015). Several methods of correcting and standardising for
haematocrit have been proposed (den Burger et al., 2015, Leuthold et al., 2015) but add further complexity to the procedure.

The advantages of using dried blood spots as a method of collecting samples for drug analysis include:

- Whole blood sample matrix
- Easy to collect, store, and transport
- A & B samples possible
- Stable, easy to handle and store
- Adaptable to a variety of analytical techniques
- Quality protocols already developed
- Centralised testing easily implemented
- Safety

Despite the advantages, there are nevertheless difficulties in using dried blood sampling. Regardless of whether or not a clinician is used to take the sample there is still the issue of infection by blood from individuals who have blood borne infectious diseases such as hepatitis or human immunodeficiency virus (HIV), and since the skin must be pierced, there is the potential risk of infection at the sampling site. Added to which there is always the risk of individuals, police staff or suspects, fainting as a result of vasovagal reactions to the sight of blood (Zervou et al., 2005). Whoever is taking the blood needs to have been trained if reliable samples are to be taken onto absorbent paper. The disadvantages of using dried blood spots as a method of collecting samples for drug analysis include:

- Skin puncture required;
- Low but potential risk of infection;
- Small sample volume;
- Variable haematocrit;
- Dilution for analysis
- Suitability for analytical methods
- Inaccuracy and imprecision
- Standardisation

In the future some of these disadvantages may be overcome by using commercial collection devices. One such device is the HemaSpot™-HF blood collection device from Spot On Sciences (Spot On Sciences, 2016b). The device is a cartridge containing absorbent paper and desiccant covered with an application surface that contains a small opening to allow for the entry of the blood. After a finger stick by lancet, two drops of
blood are applied, the cartridge is closed. The desiccant rapidly dries the sample. The sample is immediately ready for shipping or storage while the moisture-tight cartridge and tamper-resistant latch assures the sample remains uncompromised. The device is approximately the size of a credit card and about 1 cm tall when closed. The manufacture claims that the “HemaSpot™-HF allows anyone to take a blood sample at any time and any location”. So far the device appears only to have been used to collect blood for testing of infections (Rosypal et al., 2014, Brooks et al., 2016).

7.2 SMALL LIQUID SAMPLES
In their simplest form these take the form of either a small volume of blood sampled into a container, often then diluted before storage, or blood sampled into a capillary, with, or without, washout and dilution before storage. Both methods suffer from the same disadvantages as dried blood spot sampling and have few of the advantages. Nevertheless small liquid samples can, and have been, used successfully for the measurement of drugs and give comparable accuracy and precision to large volume sampling (Merton et al., 2000).

As with dried blood spot sampling, in the future some of these disadvantages may be overcome by using commercial collection devices. One such device in development is the “revolutionary, yet absurdly simple dried matrix microsampling device” Mita™ (Phenomenex, 2016). This is a volumetric absorptive microsampling device that accurately and precisely (coefficient of variation, CV≈ 4 %) samples 10 µL of blood following a fingertip lancet stab. Although a skin puncture is still required the device eliminates most of the other disadvantages associated with dried blood spot sampling while maintaining all of the advantages of DBS sampling. In tests the results from device has been shown to overcome the issues associated the variations in blood haematocrit (Spooner et al., 2015) and to give reproducible results for drug assays (Denniff et al., 2015).

7.3 DBS AND SMALL LIQUID SAMPLES - SUMMARY
As yet the procedures and technology for either dried blood spot sampling or small liquid samples has not progressed far enough to be used in an environment such as a police station or at the road side. However there are developments of commercial devices that may result in the near future in devices that would be suitable for use in sampling suspected drug-drivers blood by law enforcement officers.
8 LATENT FINGERPRINTS

The traditional matrices for the detection of drug use are blood, hair, urine and OF. Drugs and their metabolites have also been detected in sweat sampled using patches or wipes (Mali et al., 2011, Kidwell et al., 1998) but, most recently it has been shown that drugs and drug metabolites can be detected in the sweat deposited in a single fingerprint (Leggett et al., 2007, Hazarika et al., 2008).

The prospect of using fingerprints as a matrix for drug detection is attractive because fingerprints can be rapidly collected in a non-invasive and straightforward manner, making them highly applicable to all avenues of drug testing, from roadside and workplace testing, through to sports anti-doping tests and forensic detection. Fingerprints are easy to handle, transport and store and the likelihood of adulterated samples would be rare (Kuwayama et al., 2015).

8.1 RESIDENCE AND DETECTION TIME OF DRUGS IN FINGERPRINTS

There are two possible routes by which latent fingerprints can become contaminated with a drug. The first of these is through contact transfer, when the fingertip comes into contact with a drug and transfers the drug to the next thing it touches. The second route for contamination of fingerprints is a direct result of drug consumption, whereby the parent drug and its major metabolites are excreted onto the skin through the action of sweating (Kidwell et al., 1998).

8.2 COLLECTION METHODS AND DEVICES USED FOR FINGERPRINT COLLECTION

Sweat from latent fingerprints has been used to detect different drugs of abuse. Several techniques have been used. For instance, FTIR and Raman spectral imaging have been used to image a latent fingerprint and detect exogenous substances deposited within it. These methods successfully detected aspirin, diazepam and caffeine that had been deposited together in another fingerprint (Ng et al., 2009).

A hydrophobic silica dusting agent containing carbon black has also been used with latent finger marks to demonstrate that the agent can act as an enhancing matrix to generate a simple method for detecting a range of drugs using surface assisted laser desorption/ionisation time-of-flight mass spectrometry (SALDI-TOF-MS). This method has been applied to the analysis of latent finger-marks for contact residues on fingers, and for detection of illicit drugs for both parent drugs and their metabolites using SALDI-TOF-MS-MS. The distribution of these compounds on fingerprints has been demonstrated using commercially available imaging software (Rowell et al., 2009).
In addition, an immunoassay based technique using magnetic particles functionalized with anti-morphine and anti-benzoylecgonine antibodies were used for the detection of morphine and BZE; detected individually as well as simultaneously from a single fingerprint (Hazarika et al., 2010). Similarly, excreted metabolites of drugs of abuse have been detected in fingerprints using ambient mass spectrometry. Paired fingerprints and OF were analysed for the presence of cocaine and benzoylecgonine using Desorption Electrospray Ionization (DESI) and Ion Mobility Tandem Mass Spectrometry Matrix Assisted Laser Desorption Ionization (MALDI-IMS-MS/MS) and GC-MS, respectively. The detection of cocaine, benzoylecgonine (BZE) and methyl-ecgonine (EME) in latent fingerprints using both DESI and MALDI showed good correlation with OF testing (Bailey et al., 2015).

8.3 LATENT FINGERPRINTS - SUMMARY

The analysis of drugs in latent fingerprints is an exciting new development that shows promise in a number of arenas that require flexible drug screening services. The Panel noted that quantitative analysis of drugs of interest is not currently well developed and therefore could not recommend the use of latent fingerprints as an alternative to blood for evidential testing. Consideration will need to be given to the issue of external contamination and how this can be negated as part of the sample collection procedure.
9 EXHALED BREATH

Although interest in analysing exhaled breath originated in the 1970s presently, the main application of the measurement of volatile components in breath relevant for the driving environment is alcohol testing. However, recent work on exhaled breath condensate (EBC) as an innovative matrix has shown that non-volatile compounds are present in breath. Low molecular weight endogenous compounds, as well as therapeutic and illicit drugs are present as non-volatile components in human breath (Popov, 2011).

9.1 COLLECTION METHODS AND DEVICES USED FOR EXHALED BREATH COLLECTION

EBC is a sampling method that collects volatile, non-volatile and condensed water from exhaled breath. In this technique a cold trap is used to collect whatever comes out in the exhaled breath after passing the oral cavity (Kuban and Foret, 2013). The resulting condensate is a water solution consisting mainly of condensed water vapour mixed with other volatile and non-volatile components. A sampling time of 10 min is often applied and more sophisticated stationary as well as portable instruments are being used for the collection procedure.

Conclusions about the effectiveness of EBC as a sampling technique for drug testing are difficult to make because the collection of EBC has not been well evaluated for this application. Commercial sampling systems are now available however and are being used to screen for drugs of abuse in research studies (Kuban and Foret, 2013, Konstantinidi et al., 2015). Stationary instruments have in-built cooling systems and may separately collect the EBC fraction that carries biomarkers from the dead space of the instrument. Portable collectors are dependent on external cooling before sampling can take place. The introduction of EBC testing in a routine manner has been delayed because of the lack of standardized measures for sample collection, which has been suggested as a major limitation (Konstantinidi et al., 2015).

Other techniques (impaction) have been used to collect aerosol fractions from EBC according to particle size. A system for collecting EBC particles in this way has been demonstrated (Almstrand et al., 2009) and a collection system is now commercially available (PeXA, 2016).

A cannabis "breathalyser" using differential mobility spectrometry (DMS) is under commercial development (Weise, 2016). This should not require the collection of exhaled breath condensate as a specific sample but whether the equipment can be reduced in size for hand held use remains to be seen (Roscioli et al., 2014).
Ellefsen (Ellefsen et al., 2014) found few breath specimens positive after intravenous dosing with 25mg cocaine. They collected breath from 10 healthy adults after administration on three separate days using a ‘SensAbues’ device (Sensabues, 2016). With a LOQ of 25 pg/filter only 2.6 % of samples were positive for cocaine, 0.72 % for benzoylecgonine and 0.72 % for EME. They concluded that detection of cocaine in breath identifies recent ingestion but that absence does not preclude recent use.

9.2 Breath - Summary
The Panel felt that at the time of writing this matrix was not yet suitable for confirmatory testing those apprehended for drug-driving offences.
10 National and International Approaches to Drug Driving

Drug-driving is a safety concern of increasing importance in the United States and in Europe (Asbridge et al., 2014, Wolff and Johnston, 2014, Wolff et al., 2013). In the United States 10.3 million individuals aged 12 years or older operated a motor vehicle under the influence of illicit drugs in 2011 (SAMHSA, 2014). Driving under the influence of drugs has been identified as a priority area for drug control research and interventions by the US Office of National Drug Control Policy and the Department of Transportation (Wong et al., 2014). In 2013, 28% of people surveyed in England and Wales thought that drug-driving was one of the top three road safety issues that should be addressed (BRMB, 2013).

Several approaches have been taken internationally to manage those who drive under the influence of psychoactive substances. Setting a concentration threshold for a psychoactive drug in relation to road traffic legislation has been implemented across Europe and wider afield. Some countries have instigated a programme of zero tolerance, which equates to a complete ban on the use of a specified drug whilst driving. Limits may be set at the laboratory limit-of-detection (LOD). This is the lowest concentration of the drug that the analytical procedure can reliably differentiate from a concentration of zero and can be positively identified according to predetermined criteria or levels of statistical confidence. When the LOD is applied as a cut-off for drug-driving purposes it is often termed the zero tolerance approach. The cut-off limits for the zero tolerance approach are thus analytical and may be set nationally or regionally (by State) and are required to be observed by the laboratory.

Table 29 LOD cut-offs from ROSITA (Verstraete and Raes, 2006) and DRUID (DRUID, 2012) for illicit drugs

<table>
<thead>
<tr>
<th>Substance</th>
<th>ROSITA Blood Conc (µg/L)</th>
<th>ROSITA OF Conc (µg/L)</th>
<th>DRUID Blood Conc (µg/L)</th>
<th>DRUID OF Conc (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>20</td>
<td>25</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>MDMA</td>
<td>20</td>
<td>25</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Cocaine</td>
<td>20</td>
<td>4</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>BZE</td>
<td>20</td>
<td>4</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>6-MAM</td>
<td>2</td>
<td>2</td>
<td>10</td>
<td>5*</td>
</tr>
</tbody>
</table>

*(Vindenes et al., 2012b)

Laboratory limit of detection levels have been suggested for whole blood and OF (Logan et al., 2013) and were published following the ROSITA and the DRUID studies (DRUID,
2012, DRUID, 2011, Verstraete and Raes, 2006) and those drugs relevant to the UK drug-driving legislation are reported for illicit drugs in Table 29 and for medicinal drugs in Table 30.

The impairment method (where a test is employed to judge a driver’s level of impairment at the road-side) requires the prosecutor to prove that the drug impaired the driver’s ability to operate a motor vehicle (Grotenhermen et al., 2007). This approach exists in England and Wales, Road Traffic Act 1988 Section 4 (UK Government, 1988), and in many EU countries legislation exists to allow prosecution of someone who is driving while unfit (impaired) through drink or drugs.

Impairment testing is implemented in many of the States of North America. It is acknowledged that impairment testing is difficult and complex to enforce, particularly due to the lack of standardization in methods of assessing and determining drug-induced impairment (Wolff et al., 2013, Romano and Pollini, 2013). As a result, in comparison to drink driving, drug-driving is prosecuted less often when impairment is used alone for evidential purposes and many jurisdictions are moving towards per se testing either in combination with impairment or as a separate offence (DuPont et al., 2012).

Table 30 Laboratory Drug Testing Parameters (Cut-off) from ROSITA (Verstraete and Raes, 2006) and DRUID (DRUID, 2012) for medicinal controlled drugs.

<table>
<thead>
<tr>
<th>Substance</th>
<th>ROSITA Blood Conc (µg/L)</th>
<th>ROSITA OF Conc (µg/L)</th>
<th>DRUID Blood Conc (µg/L)</th>
<th>DRUID OF Conc (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>20</td>
<td>25</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Morphine</td>
<td>10</td>
<td>20</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Methadone</td>
<td>20</td>
<td>20</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Diazepam</td>
<td>50</td>
<td>0.5</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>50</td>
<td>0.5</td>
<td>50</td>
<td>5</td>
</tr>
<tr>
<td>Temazepam</td>
<td>50</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lorazepam</td>
<td>50</td>
<td>0.5</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>50</td>
<td>0.2</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Flunitrazepam</td>
<td>2</td>
<td></td>
<td>1*</td>
<td></td>
</tr>
</tbody>
</table>

* (Vindenes et al., 2012a)

The ‘per se’ approach is based on the detection of a drug in a driver above a defined cut-off concentration predominantly in whole blood and cut-off concentrations have been linked to risk and driver safety or deemed to be equivalent to a drug concentration that
causes a pharmacological effect. Implementation of the ‘per se’ threshold has been interpreted in several different ways.

A threshold can be technical and can refer to the laboratory limit-of-quantification (LLOQ). This is defined as the lowest measurable quantity of a drug that can be detected according to the technological limits of the equipment with an acceptable level of accuracy and precision and that guarantees a valid and reliable analytical determination of the drug of interest. The LLOQ is usually set above the LOD.

A Home Office expert working group led by CAST during 2013-14 agreed that blood concentrations could not be correlated with those in OF so the ‘risk threshold’ limits set in the Section 5A legislation for medicinal controlled drugs could not be translated into OF cut-offs (UK Government, 2014).

It was noted that since drugs can exert a pharmacological effect at very low concentrations in the body cut-off levels are typically in the order of µg/L. Per se drug drive limits reflect this, and are many orders of magnitude lower than those for drink driving; typical limits may be in the order of nano-grams per millilitre, in comparison to several milli-grams per hundred milli-litres of whole blood for alcohol. ‘Pharmacological effect’ cut-offs in OF were agreed alongside LLOQ cut-offs. The latter were published in ‘A Guide to Type Approval Procedures for Preliminary Drug Testing Devices Used for Transport Law Enforcement in Great Britain’ (Home Office, 2012).

The pharmacological effect cut-off concentrations in OF were set using the same rationale as the LLOQ cut-offs for blood, i.e. the lowest detectable amount that, in the opinion of the working group, most laboratories would be able to detect, yet above the concentrations commonly associated with passive exposure (Table 31). They are closely aligned with those referenced in the DRUID report (DRUID, 2012).
Table 31  Thresholds estimated in OF for both a ‘pharmacological effect and for a low cut-off concentration (µg/L) as determined by the CAST expert working group.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Pharmacological Effect, (µg/L)</th>
<th>LLOQ (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>Methylene dioxyethylamphetamine (MDMA)</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>Ketamine</td>
<td>Na</td>
<td>20</td>
</tr>
<tr>
<td>Cocaine</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Benzoylecgonine (BZE)</td>
<td>As a composite</td>
<td>30</td>
</tr>
<tr>
<td>Morphine</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>6-monoacetyl-morphine (6-MAM)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Methadone</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Diazepam</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Temazepam</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

A ‘lower effect threshold’ limit has also been described and is usually equivalent to a blood alcohol concentration (BAC) of 0.2 g/L (20 mg/dL, 0.02 %) alcohol. In 2012 legal limits in whole blood for twenty illegal drugs and medicines with an abuse potential were introduced in Norway. *Per se* limits corresponding to blood alcohol concentrations (BAC) of 0.2 g/L were established for 20 psychoactive drugs, and limits for graded sanctions corresponding to BACs of 0.5 and 1.2 g/L were determined for 13 of these drugs.

The new legislation has made it possible for the courts to determine the offence based on the laboratory test results. The impairment limits and limits for graded sanctions can
be found in the tables below (Norwegian Ministry of Transport and Communications, 2014).

Table 32  *Per Se* legal limits in traffic in Norway for illicit drug (NIPH, 2016)

<table>
<thead>
<tr>
<th>Substance</th>
<th><em>Per se</em> limit equivalent to 0.2 g/L whole blood µg/L (µmol/L)</th>
<th>Limits for graded sanction equivalent to 0.5g/L whole blood µg/L (µmol/L)</th>
<th>Limit for graded sanction equivalent to 1.2g/L whole blood µg/L (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC</td>
<td>1.3 (0.004)</td>
<td>3.1 (0.01)</td>
<td>9.4 (0.03)</td>
</tr>
<tr>
<td>Cocaine</td>
<td>24.3 (0.08)</td>
<td>^</td>
<td>^</td>
</tr>
<tr>
<td>MDMA</td>
<td>96.6 (0.5)</td>
<td>^</td>
<td>^</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>40.56 (0.300)</td>
<td>^</td>
<td>^</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>44.8 (0.3)</td>
<td>^</td>
<td>^</td>
</tr>
<tr>
<td>Ketamine</td>
<td>46.9 (0.2)</td>
<td>237.7 (0.5)</td>
<td>285.3 (1.2)</td>
</tr>
<tr>
<td>LSD</td>
<td>0.97 (0.003)</td>
<td>^</td>
<td>^</td>
</tr>
</tbody>
</table>

*Legal limits are presented in micromoles/L in Norway and have been converted to µg/L for ease of understanding. ^Legal limits were not defined as the relationship between blood concentration and driving skills are highly variable or poorly documented in scientific literature.

In 2015 the legal limits in Norway were revised and in February 1, 2016 an additional eight substances were added to the legislation. *Per se* limits for a total of 28 different illegal drugs and medicines were established; 22 for graded sanctions equivalent to BACs of 0.5 g/L and 1.2 g/L. Legal limits and cut-off for graded sanctions currently used in Norway are shown in Table 32 for illicit drugs and Table 33 for medicinal controlled substances.
### Table 33  
**Per Se legal limits in traffic in Norway for medicinal drugs (NIPH, 2016)**

<table>
<thead>
<tr>
<th>Substance</th>
<th><em>Per se</em> limit equivalent to 0.2 g/L whole blood µg/L (µmol/L)</th>
<th>Limits for graded sanction equivalent to 0.5 g/L whole blood µg/L (µmol/L)</th>
<th>Limit for graded sanction equivalent to 1.2g/L whole blood µg/L (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>8.57 (0.0300)*</td>
<td>22.86 (0.080)</td>
<td>57.14 (0.200)</td>
</tr>
<tr>
<td>Methadone</td>
<td>24.8 (0.08)</td>
<td>61.9 (0.20)</td>
<td>148.5 (0.48)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>56.9 (0.20)</td>
<td>142.4 (0.50)</td>
<td>341.6 (1.20)</td>
</tr>
<tr>
<td>Flunitrazepam</td>
<td>0.31 (0.005)</td>
<td>3.1 (0.01)</td>
<td>7.8 (0.025)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>1.3 (0.004)</td>
<td>3.2 (0.10)</td>
<td>7.9 (0.025)</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>9.6 (0.03)</td>
<td>24.1 (0.075)</td>
<td>57.8 (0.18)</td>
</tr>
<tr>
<td>Nitrazepam</td>
<td>16.9 (0.06)</td>
<td>42.2 (0.15)</td>
<td>98.5 (0.35)</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>172 (0.6)</td>
<td>430 (1.5)</td>
<td>860 (3.0)</td>
</tr>
</tbody>
</table>

*Legal limits are presented in micromoles/L in Norway and have been converted to µg/L for ease of understanding.*

A *per se* threshold can also relate to risk and refer to a drug concentration cut-off set in whole blood indicating a certain crash risk associated with driving under the influence of a drug above that threshold. ‘Risk thresholds’ for instance, have been determined showing the same level of accident risk as a BAC of 50 mg/dL (0.5 g/L) alcohol blood (DRUID, 2012). From the point of view of setting thresholds in a biological fluid, reference values (concentration/effect ratios) are more readily available for blood (plasma or serum) than OF. For establishing thresholds in the context of drug-driving legislation based on driving performance, blood is the gold standard since it is generally well described in the scientific literature and is best related to behavioural effects on driving (Wille et al., 2009).

Estimating the risk of a driver’s involvement in road traffic collisions (RTC) has often been used to determine the impact on road safety of whose driving under the influence of alcohol or drugs. In drug-driving research, calculation of the odds ratio (OR) or the relative risk (RR) ratio involves the comparison of two groups of drivers (e.g. drug driver versus non-drug driver) and gives an indication of the likelihood of a RTC happening to the one group compared to the other (Davies et al., 1998); two levels of road collision risk (being...
a fatality or being seriously injured) are usually considered (Ravera et al., 2012, NFI, 2010). The European study DRUID has classified ORs as “low risk” (OR <2.0), “medium risk” (OR >2.0 – 10.0) and “high risk” (OR >10.0) (DRUID, 2012). In the DRUID case-control study the risk of being seriously injured or killed was calculated against control data from the roadside survey and case data from the hospital study on killed drivers. The risk estimates (odds ratios) were adjusted for age and gender; the controls were weighted with traffic distribution in eight time periods over a week.

The use of ‘specified limits’ or a ‘per se’ approach has been introduced for 17 compounds contained within Section 5A of the Road Traffic Act 1988, which came in to force in March 2015. The limits (in whole blood) are set out in ‘The Drug Driving (Specified Limits) (England and Wales) Regulations 2014’ (UK Government, 2014) as shown in Table 34.

Table 34  
Cut-off levels specified in the drug driving (section 5A) offence
(UK Government, 2014)

<table>
<thead>
<tr>
<th>Illicit Compounds</th>
<th>Blood Concentration µg/L</th>
<th>Medicinal compounds</th>
<th>Blood concentration µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC</td>
<td>2</td>
<td>Amphetamine</td>
<td>250</td>
</tr>
<tr>
<td>Cocaine</td>
<td>10</td>
<td>Morphine</td>
<td>80</td>
</tr>
<tr>
<td>BZE</td>
<td>50</td>
<td>Methadone</td>
<td>500</td>
</tr>
<tr>
<td>Ketamine</td>
<td>10</td>
<td>Diazepam,</td>
<td>550</td>
</tr>
<tr>
<td>LSD</td>
<td>1</td>
<td>Oxazepam</td>
<td>300</td>
</tr>
<tr>
<td>Meth-amphetamine</td>
<td>10</td>
<td>Temazepam</td>
<td>1000</td>
</tr>
<tr>
<td>MDMA</td>
<td>10</td>
<td>Clonazepam</td>
<td>50</td>
</tr>
<tr>
<td>6-MAM</td>
<td>5</td>
<td>Flunitrazepam</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lorazepam</td>
<td>100</td>
</tr>
</tbody>
</table>

For those drugs which have widespread medicinal use, limits are set at a concentration at which there is evidence of an increased risk of road traffic collision. Steady-state drug concentrations when used therapeutically were also taken into consideration (Wolff et al., 2013). Limits for illicit drugs in the offence were set on the basis of a LLOQ approach,
taking in to account any applicable risk of a drug being present through accidental contamination or passive exposure (CAST, 2014). An offence is committed if the concentration of the drug in the driver’s body is in excess of the limit specified for that drug. Punishments include a fine (£5000), points on the driving licence, removal of a driving licence and imprisonment.

10.1 COUNTRY SPECIFIC THRESHOLDS
At the WHO second technical consultation on ‘Drug Use and Road Safety’ (WHO, 2015) the Netherlands presented their new approach to drug-driving and proposed the use of OF for road-side ‘preselection tests for drugs’ (spring 2016) and expect to introduce legal limits for illicit drugs in whole blood (summer 2016). The Netherlands also plan to introduce a revised cut-off limit for morphine (20 µg/L) and GHB (10 mg/L) as part of their new legislation.

In Germany it is a regulatory offence (StVG paragraph 24a, 2, Road Traffic Law) to drive a vehicle while under the influence of cannabis, heroin (morphine), cocaine (BZE), amphetamine, methamphetamine and MDMA. Individuals are deemed to be under the influence if one of the substances is detected in blood (Steinmeyer et al., 2001). There is an exemption if it is as a consequence of taking prescribed medication for a specific medical condition, such as in the UK. There are no limits for drugs used for medical conditions however, so that any amount of any of the banned substances is sufficient. Indicative limits have been produced and also include a cut-off for morphine at 10 µg/L in blood. If any impairment is observed in addition to the presence of the drugs, this constitutes a possible road traffic offence (the decision as to what charge is made depends on whether the person is thought to have caused a danger to other road users).

For a regulatory offence the penalty is a fine of at least 250 Euros, and a driving ban of 1 month. Some federal states in Germany also require a medical and psychological examination before the driving licence is returned which the offender has to pay for and which costs around 500 Euros. If there are previous road traffic offences the penalty may be increased to 1500 Euros and a ban of up to 3 month as well as 4 penalty points.

In Spain, any driver may be required to take part in a roadside drug or alcohol test as a random precautionary check or following a RTC, when a police officer is responding to a complaint or if a driver displays impairment. There is a zero tolerance approach to illegal drugs. The drug-driving legislation is separated into administrative proceedings (a fine of 1000 Euros and 6 points on the driving license) or criminal proceedings; imprisonment for 3-6 month, 6-12 month penalty, 30-90 days community service or driving disqualification from 1 to 4 years.
The roadside screening test in Spain uses OF to detect THC, cocaine, amphetamine, methamphetamine and morphine. Both the Dräger Drugtest® 5000 and Alere DDS2 are used by police officers. The confirmatory evidence test requires a second OF sample for laboratory analysis in order to confirm the initial screening test result. A blood sample may also be provided. The Guardia Civil Traffic Department have performed 105 000 tests in Spain since 2011 and have found 35% drivers to be positive for an illicit substance.

In Australia a Random Drug Testing (RDT) programme is conducted in various locations across metropolitan Melbourne and regional areas in Victoria. The location and time of the RDT operation is decided by the police based on local intelligence. There are three potential target groups chosen in order to maximise detection. These groups are motorists in areas of high drug use prevalence, high-risk drug users associated with the road transport industry and high-risk drug users associated with the ‘dance and rave’ setting. Confirmation cut-offs in Australia in OF are 5 µg/L for methamphetamine and MDMA and 2 µg/L for THC.

Drug testing is conducted by randomly intercepting small groups of drivers from the traffic passing through the police checkpoints. There are three stages to the testing procedure. First, a preliminary breath test for alcohol is conducted. The current BAC limit in Australia for a full licensed driver is 0.05 g/100mL and a zero limit for drivers holding a commercial or probationary license. The driver is not further tested for the presence of a drug if a positive test for alcohol is confirmed. For those drivers who test negative for alcohol, a preliminary drug test is conducted, screening for THC, methamphetamine and MDMA in OF using DrugWipe® II. Drivers with a positive test are required to provide 1 mL of OF sample for the second test in a police vehicle using a Cozart® device (Alere™, 2016a), which collects 1 mL OF that is diluted to 3 mL with a buffer. An aliquot of this diluted sample is analysed in an instrument called Rapiscan®, which indicates the presence of drugs electronically. Blood specimens are collected from individuals unable to provide adequate OF. Those drivers who are tested positive following a Cozart/Rapiscan test are prohibited from driving for 4 to 12 hours, depending on the detected substance. These drivers will be formally prosecuted once the evidential laboratory tests confirm the presence of the prescribed drugs in OF or blood (WHO, 2016).

10.2 Published limits for illicit drugs included in drug-drive legislation
It is important to note that the limits set in other countries need to be considered alongside their legal system and the specific drug driving legislation. Some countries have set very low limits, which are often referred to as a zero-tolerance approach, but they may use these limits in conjunction with an impairment-type drug driving offence, where the sanctions apply only if impaired driving is also recorded e.g. as in Germany.
10.2.1 Cannabis

It is widely acknowledged that cannabis is the most commonly used illegal drug and this prevalence carries over into drug-driving populations, with notable exceptions (Freeman et al., 2011, Gjerde et al., 2011b, Chu et al., 2012). The blood-concentration-time profile of THC shows a significant dose effect for cannabis and driving performance. This relationship has been observed in simulated and real-life situations, in which raised concentrations of THC were associated with increased traffic crash risk (Jones et al., 2008b, Mura et al., 2003, Khiabani et al., 2006, Grotenhermen et al., 2007).

To this end cannabis (or more specifically THC) has been enshrined in drug-driving legislation across Europe and beyond (Table 35). THC is the only cannabis compound currently recommended for OF screening and is the only substance recommended by SAMHSA, with a cut-off of 2 µg/L and DRUID who set a cut-off of 1 µg/L, for OF confirmation tests (SAMHSA, 2015) and (DRUID, 2011).

Table 35 International drug thresholds (set in or recommended for legislation) for THC

<table>
<thead>
<tr>
<th>Country</th>
<th>Approach to threshold</th>
<th>THC threshold in blood (µg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>Zero tolerance</td>
<td>25 (OF) screen; 2 confirmation</td>
<td>(Boorman and Owens, 2009)</td>
</tr>
<tr>
<td>Belgium</td>
<td>Analytical cut-off</td>
<td>1.0</td>
<td>(de Gier et al., 2009)</td>
</tr>
<tr>
<td>Norway</td>
<td>Impairment limit</td>
<td>1.3, 3.0, 9.0</td>
<td>(Norwegian Ministry of Transport and Communications, 2014)</td>
</tr>
<tr>
<td>Portugal</td>
<td>Analytical cut-off</td>
<td>3.0</td>
<td>(EMCDDA, 2016)</td>
</tr>
<tr>
<td>Sweden</td>
<td>Zero tolerance</td>
<td>0.3</td>
<td>(Holmgren et al., 2008)</td>
</tr>
<tr>
<td>Switzerland</td>
<td>Prosecution Threshold</td>
<td>1.5</td>
<td>(EMCDDA, 2016)</td>
</tr>
<tr>
<td>England &amp; Wales</td>
<td>LLOQ, Strict liability offence</td>
<td>2.0</td>
<td>(UK Government, 2014)</td>
</tr>
<tr>
<td>Germany</td>
<td>Indicative</td>
<td>1.0</td>
<td>(Germany, 2003)</td>
</tr>
</tbody>
</table>

It is interesting to note that in North America where cannabis use has been de-criminalised in many states, legal limits have been set for THC in urine as well as in whole blood (Wong et al., 2014). It is also noteworthy that in Ohio there is a lower limit for cannabis when identified in combination with alcohol (Table 36).
Table 36  Legal THC thresholds for drivers in different states in North American

<table>
<thead>
<tr>
<th>State</th>
<th>Confirmatory test matrix</th>
<th>Blood (µg/L)</th>
<th>Urine (µg/L)</th>
<th>Year legislation introduced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorado</td>
<td></td>
<td>5.0</td>
<td></td>
<td>2013</td>
</tr>
<tr>
<td>Iowa</td>
<td></td>
<td></td>
<td>50.0 THC-COOH</td>
<td>2010</td>
</tr>
<tr>
<td>Montana</td>
<td></td>
<td>5.0</td>
<td>10.0 THC</td>
<td>2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15.0 THC-COOH</td>
<td></td>
</tr>
<tr>
<td>Nevada</td>
<td></td>
<td>2.0</td>
<td>10.0 THC</td>
<td>2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15.0 THC-COOH</td>
<td></td>
</tr>
<tr>
<td>Ohio*</td>
<td></td>
<td>2.0</td>
<td>10.0 THC</td>
<td>2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>35.0 THC-COOH</td>
<td></td>
</tr>
<tr>
<td>Pennsylvania</td>
<td></td>
<td>1.0 THC-COOH;</td>
<td>1.0 µg/L</td>
<td>2011</td>
</tr>
<tr>
<td>Washington</td>
<td></td>
<td>5.0</td>
<td></td>
<td>2013</td>
</tr>
</tbody>
</table>

*There is a lower cut-off when THC-COOH is detected in combination with alcohol or other drugs; 15.0 µg/L in urine, 5.0 µg/L in blood

10.2.2  Cocaine and BZE

Drug-driving legislation for cocaine has been adopted using different approaches. Cocaine is a short acting drug and the detection of cocaine alone is known to suggest immediacy of use. The detection of cocaine alongside its primary metabolite BZE is much more common and suggests use within the last 12 h. Detection of BZE alone is known to indicate cocaine use in the past and has been associated with driver sedation due to sleep exhaustion and attributed to the ‘come down’ period after cocaine use (Jones et al, 2008). Researchers have observed that when both cocaine and BZE are detected together, the BZE concentration in blood is uniformly higher than the cocaine concentration (mean cocaine concentration 836 µg/L) with a typical BZE to cocaine ratio being 14.2: 1, range 1:1 to 55:1 (Jones et al., 2008a). To this end some international drug-driving legislation including England and Wales has included cut-off limits for BZE as well as cocaine (Table 37).
Table 37  International drug thresholds set or recommended for legislation for cocaine and BZE

<table>
<thead>
<tr>
<th>Country</th>
<th>Approach to threshold</th>
<th>Cocaine (µg/L)</th>
<th>BZE* (µg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>England &amp; Wales</td>
<td>LLOQ, Strict liability offence</td>
<td>10 (B)</td>
<td>50 (B)</td>
<td>(Wolff et al., 2013)</td>
</tr>
<tr>
<td>Finland</td>
<td>Zero tolerance</td>
<td>15 (Se)</td>
<td>10 (Se)</td>
<td>(Blencowe et al., 2012)</td>
</tr>
<tr>
<td>Germany</td>
<td>Indicative</td>
<td>10 (Se)</td>
<td>75 (Se)</td>
<td>(Germany, 2003)</td>
</tr>
<tr>
<td>Netherlands</td>
<td>Threshold</td>
<td>50 (B)</td>
<td></td>
<td>(NL Advisory Committee, 2010)</td>
</tr>
<tr>
<td>Portugal</td>
<td>Zero tolerance</td>
<td>5 (B)</td>
<td>5 (B)</td>
<td>(EMCDDA, 2016)</td>
</tr>
<tr>
<td>Norway</td>
<td>Impairment limit ≈ 0.2 g/L BAC</td>
<td>24 (B)</td>
<td></td>
<td>(NIPH, 2016)</td>
</tr>
</tbody>
</table>

Key: Biological fluids; B – blood; Se – serum; OF – OF; *BZE (benzoylecgonine) is the main metabolite of cocaine

10.2.3 Ketamine

Ketamine is becoming increasingly prevalent in drug using communities and in those who drive. The CSEW indicates that 40 % of respondents admitting to driving under the influence of drugs had previous experience of ketamine intake, either alone or in combination with other drugs (CSEW, 2015). Others have specifically identified the presence of ketamine in fatal (9 % positive tests) (Cheng et al, 2005) and non-fatal driving (45 % ketamine positive tests) traffic accidents (Wong et al., 2010). In 2012, 13 of 853 OF samples taken from random roadside testing in the state of Victoria, Australia were found positive for ketamine (Chu et al., 2012). Whereas in the UK in 2013, Burch et al reported 14 ketamine-positive individuals from 376 suspected cases of driving under the influence of drugs (Burch et al., 2013). In a Dutch population (n = 3038) ketamine was detected in 10 blood samples collected from impaired drivers (Bezemer et al., 2014).

Ketamine use in UK drivers may be underestimated since it is not included in the standard FSP analytical panel and in the Burch study was only ‘looked for’ if suspected. The mean and median concentrations of ketamine in the UK dataset were 421 µg/L and 385 µg/L, respectively, and the concentrations for norketamine were 605 µg/L and 410 µg/L.

Very few jurisdictions have included ketamine in their drug-driving legislation. One exception is Hong Kong; since March 2012 drug driving legislation has allowed blood and urine specimens to be used, to provide a direct objective test to verify whether a driver had taken drugs. Before performing an intimate sample test, an "Impairment Test" is used
to first identify non-offenders and verify whether suspected drivers have taken drugs before or while driving (Hong Kong Police Force, 2015). The legal Ordinance in Hong Kong introduces a zero-tolerance offence for certain specified illicit drugs, namely heroin, ketamine, methamphetamine, cannabis, cocaine and MDMA.

Table 38 International drug thresholds set or recommended in the legislation for ketamine

<table>
<thead>
<tr>
<th>Country</th>
<th>Approach to threshold</th>
<th>Ketamine Whole blood (µg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>England &amp; Wales</td>
<td>LLOQ, Strict liability offence</td>
<td>10</td>
<td>(UK Government, 2014)</td>
</tr>
<tr>
<td>Norway</td>
<td>Low limits (µg/L) Impairment (0.5g/L BAC)</td>
<td>55</td>
<td>(Norwegian Ministry of Transport and Communications, 2014)</td>
</tr>
<tr>
<td></td>
<td>Impairment (1.2 g/L BAC)</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Impairment</td>
<td>329</td>
<td></td>
</tr>
<tr>
<td>Hong Kong</td>
<td>Impairment</td>
<td>Any amount above LOD</td>
<td>(Police, 2016)</td>
</tr>
</tbody>
</table>

10.2.4 Amphetamines

According to the United Nations Office on Drugs and Crime amphetamines are the second class of most used illicit drugs in the world. Their consumption for recreational purposes has increased significantly over the past years due to its easy availability and low cost (UNODC, 2015). Amphetamine is misused through a variety of routes, including swallowing, dissolving in a liquid and drinking, snorting (nasal insufflation) and by intravenous injection and is found in a range of forms (as ‘paste’ which is the salt form or as ‘speed’ the pure form) and purities. Users commonly ingest the drug swallowed in cigarette paper (‘bomb’) or dissolved in a drink, typically in single gram amounts (RELEASE, personal communication, www.release.org.uk/contact/).

Conversely, amphetamine is also used in the treatment of narcolepsy and for attention deficit hyperactivity disorder (ADHD), where d-amphetamine or a mixture of amphetamine salts may be used (Jenkins, 2008). Much lower doses are used for therapeutic purposes in the range of 5 – 60 mg (0.005 g - 0.06 g) per day\textsuperscript{15}.

\textsuperscript{15} It is important to note that street amphetamine is not usually pharmaceutically pure. In general terms most users expect the amphetamine content to be around 10%, although purity may be variable. This should be borne in mind when comparing therapeutic and street doses.
Studies of the effects and influence of stimulant drugs, their interaction with sleep deprivation and with alcohol on driving performance and accident risk have been conducted (Bosker et al., 2012, Hjalmdahl et al., 2012) and form the basis of legislation in Norway (Gjerde et al., 2016, Gjerde et al., 2011a) (Gjerde and Verstraete, 2010). Others used data derived from the scientific literature to determine an effective ‘active’ dose and to estimate whole blood amphetamine concentrations as a ‘soft’ guide for their legislation.

For instance, following an ‘active’ intravenous dose of 50 mg a concentration range of 50-150 μg/L was observed and on this basis a legal threshold 50 μg/L was set by the Netherlands Forensic Institute (2010). In the Netherlands and in France a common value has been set for all stimulant drugs, based on the fact that they all act in the same way (Stough et al., 2012); the legal limit thus applies collectively to amphetamine, methamphetamine, MDMA, MDEA, and MDA and the presence of one or more of any of the drugs in this class must not exceed 50 μg/L (Table 39).

MDMA and amphetamine concentrations observed in those driving under the influence are often much higher (10-fold) than those measured following controlled administration in experimental studies. For instance, Verschraagen et al (Verschraagen et al., 2007) reported (of 467 amphetamine-positive cases) that the median blood concentration of MDMA was 330 μg/L (maximum concentration detected 4,000 μg/L), whilst the median amphetamine blood concentration was 220 μg/L and ranged up to 2,300 μg/L. Clearly, much higher than amphetamine concentrations that are achieved in controlled studies (Bosker et al., 2012) and through medicinal use (Baselt, 2008).
Table 39  International drug thresholds (set in or recommended for legislation) in whole blood for amphetamine, methamphetamine and MDMA

<table>
<thead>
<tr>
<th>Country</th>
<th>Approach to threshold</th>
<th>Amphetamine (µg/L)</th>
<th>Methamphetamine (µg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netherlands</td>
<td>Threshold</td>
<td>50 *</td>
<td>50 *</td>
<td>(NL Advisory Committee, 2010)</td>
</tr>
<tr>
<td>France</td>
<td>Threshold</td>
<td>50 *</td>
<td>50 *</td>
<td>(Mura et al., 2003)</td>
</tr>
<tr>
<td>Germany</td>
<td>Indicative</td>
<td>25 *</td>
<td>25 *</td>
<td>(Germany, 2003)</td>
</tr>
<tr>
<td>England/Wales</td>
<td>Therapeutic Threshold</td>
<td>250</td>
<td>10^</td>
<td>(UK Government, 2014)</td>
</tr>
<tr>
<td>Norway</td>
<td>Impairment limit</td>
<td>41 legal limits for graded sanctions not defined</td>
<td>45 legal limits for graded sanctions not defined</td>
<td>(NIPH, 2016)</td>
</tr>
</tbody>
</table>

* The sum of the concentration of amphetamine, plus methamphetamine, plus MDMA, plus MDEA, plus MDA must not exceed 50 µg/L.  
^ Cut-off for MDMA 10 µg/L

Key; NAC Netherlands Advisory Committee, NIPH, Norwegian Institute of Public Health

Data from CAST reflects high blood amphetamine concentrations in drivers; analysis of blood samples (193/2995) taken between January 2008 and October 2012, predominantly from England and Wales, in suspected drug drive cases showed median and mean concentrations of amphetamine 270 µg/L and 456 µg/L respectively. In a later dataset Burch et al, (Burch et al., 2013) reported median and mean blood amphetamine concentrations of 360 µg/L and 496 µg/L There is evidence in Norway at least that drivers apprehended by the police for suspected drug-driving represent a somewhat marginalised group of experienced drug users with frequent re-arrests (Christophersen et al., 2002) and high mortality (Skurtveit et al., 2002).

It is widely acknowledged that stimulants are generally safe for driving when taken alone at regular doses (e.g., as in medicinal use), but amphetamine-type drugs are less safe when taken in combination with alcohol (Ramaekers et al., 2012, Strand et al., 2016). It is also well recognised that sleep loss associated with amphetamine use impairs driving performance. Taking these factors into consideration in a threshold of 250 µg/L was
specified in the legislation for amphetamine in England and Wales, whereas a LLOQ cut-off was employed for methamphetamine and MDMA (Table 39).

10.3 Published limits for psychoactive medicines

10.3.1 Opiates and opioids

An important issue for opiate/opioid drugs measured in biological fluids for drug-driving purposes is the significant overlap between heroin (diacetylmorphine) use as an illicit drug and its use alongside morphine (its primary metabolite) as a medicine. The problem is confounded by the fast acting nature of heroin, which renders detection in any biological matrices difficult. The consensus for fast acting drugs has been to include metabolites in the legislation (DRUID, 2011). However, this has led to different approaches being taken.

10.3.2 Morphine

Some international jurisdictions have used a zero tolerance approach for morphine (Table 40), whilst England and Wales have taken a more pragmatic approach setting LLOQ cut-off for the intermediary metabolite 6-MAM and a per se threshold concentration for morphine. In Denmark all illegal and legal drugs with abuse potential (e.g. opioids) are forbidden whilst driving above a fixed concentration limit in whole blood (Table 40). However, as in England and Wales, medicinal drugs are permitted if the driver has a prescription and is judged able to drive in a safe manner (investigated by a medical doctor). For therapeutic drugs such as morphine, the limits were selected as the lower therapeutic limits taken from Schulz & Schmoldt (Schulz and Schmoldt, 2003).

<table>
<thead>
<tr>
<th>Country</th>
<th>Legislative Framework</th>
<th>Morphine Cut-off (µg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belgium</td>
<td>Impairment and zero tolerance</td>
<td>10 (plasma/serum)</td>
<td>(DRUID, 2011)</td>
</tr>
<tr>
<td>Italy</td>
<td>LLOQ</td>
<td>5</td>
<td>(EMCDDA, 2016)</td>
</tr>
<tr>
<td>Poland</td>
<td>LLOQ</td>
<td>20</td>
<td>(DRUID, 2011)</td>
</tr>
<tr>
<td>Denmark</td>
<td>Impairment</td>
<td>10</td>
<td>(Berghaus, 2007)</td>
</tr>
<tr>
<td>England &amp; Wales*</td>
<td>Per se threshold</td>
<td>80</td>
<td>(UK Government, 2014)</td>
</tr>
</tbody>
</table>

England and Wales also have a LLOQ cut-off for 6-MAM set at 5 µg/L
10.3.3 Methadone
A group of international experts met in Taillores, France to propose guidelines for drug driving research (Walsh, 2009) and established a cut-off of 20 µg/L in OF for methadone, which was also adopted by the DRUID studies (Pil et al., 2010). With this criterion Gjerde et al identified 0.03 % of randomly selected drivers in Norway had driven under the influence of the drug (Gjerde et al., 2008). In England and Wales, cut-offs have been established in whole blood and reflect supratherapeutic concentrations in recognition of the need to take into consideration those stable on methadone and rehabilitated into the community.

10.3.4 Benzodiazepines
In many European countries, including the UK, benzodiazepines are the most common medicines detected in drivers. Epidemiological Studies (Barbone et al., 1998, Orriols et al., 2011, Gjerde et al., 2011b, Gjerde and Verstraete, 2010, Vermeeren, 2004, Elvik, 2013) indicate a major increase in the consumption of psychoactive medicines of this nature and this was reported in the DRUID studies, deliverable 2.2.3 and 2.2.5 (DRUID, 2012). The risk of driver impairment has been shown to increase significantly with increasing benzodiazepine blood concentration. Odds of being assessed impaired have been published for many benzodiazepines; for diazepam OR: 1.61 (P = 0.001), for oxazepam OR: 3.65 (P = 0.05) and for flunitrazepam OR: 4.11 (P = 0.05) (Bramness et al., 2002). However, the benzodiazepines have not been widely included in drug-driving legislation (Table 41).
Table 41  International drug thresholds (set in or recommended for legislation) for common benzodiazepines in whole blood

<table>
<thead>
<tr>
<th>Country</th>
<th>Approach to threshold</th>
<th>Diazepam (µg/L)</th>
<th>Oxazepam (µg/L)</th>
<th>Flunitrazepam (µg/L)</th>
<th>Clonazepam (µg/L)</th>
<th>Lorazepam (µg/L)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>England/Wales</td>
<td>Risk (therapeutic)</td>
<td>550</td>
<td>300</td>
<td>300</td>
<td>50</td>
<td>100</td>
<td>(UK Government, 2014)</td>
</tr>
<tr>
<td></td>
<td>Threshold</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Strict liability</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>offence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>Impairment limit</td>
<td>57</td>
<td>172</td>
<td>16</td>
<td>13</td>
<td></td>
<td>(Norwegian Ministry of Transport and Communications, 2014)</td>
</tr>
<tr>
<td></td>
<td>(0.2g/L BAC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>Graded Sanction</td>
<td>143</td>
<td>430</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>comparable to</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.5g/L BAC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>Graded sanction</td>
<td>342</td>
<td>860</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>comparable to</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.2g/L BAC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
10.4 Drug use combinations

It is becoming increasingly well known that drivers who misuse psychoactive substances may take more than once substance before driving. According to the Crime Survey for England and Wales (CSEW) the most common pairing of drugs used together was cannabis and powder cocaine, or cannabis and ecstasy, with these two pairings being used together in 31 per cent of all cases of simultaneous polydrug use. This also includes cases where all 3 drugs were used together (CSEW, 2015). There is also the use of alcohol with other substances, highlighted in the Technical report ‘Driving under the influence of drugs’ (Wolff et al., 2013) and is an emerging trend. In some regions (Ohio, USA) the combined use of different drugs (cannabis and alcohol) has been recognised as an increased danger to driver safety and cut-offs have been lowered compared with when each drug is detected alone (Table 36).

The Panel agreed that some discussion is needed with regard to the approach taken when more than one substance is detected in the evidential sample and particularly, whether consideration should be given to substances with a known impairing effect that are present below the level currently set in legislation but may in combination with other psychoactive substances be a risk to driver safety.

10.5 Other drugs

There is growing awareness that drugs with similar pharmacological mechanisms of action to those included in the section 5A legislation but, which are not controlled, pose similar impairing effects on driving performance. There is often incomplete knowledge of these compounds and especially, insufficient evidence regarding their possible contribution to traffic accidents. Evidence is also emerging with regard to driver safety for some drugs controlled under the Misuse of Drugs Act (1971) and these compounds have been included in drug driving legislation elsewhere. Gamma-hydroxybutyrate (GHB) with sedative and anaesthetic effects is a good example.

Estimates of the prevalence of GHB use in adult populations are much lower than for the misuse of cocaine or ecstasy but in targeted surveys among visitors to large-scale parties in the Netherlands (2009) a prevalence of GHB of 4.6 % was reported (UK Focal Point on Drugs, 2011).

10.6 Summary

A brief review of drug-driving legislation internationally has shown that individual countries take different approaches to roadside drug testing, both from a legal standpoint and from the analytical approach. The number of drugs targeted differs according to
national prevalence, although the POCT OF test is commonplace as a road-side drug screening tool. The zero tolerance (LOD) or LLOQ approach seems to be the consensus for illegal drugs, in some cases with the additional requirement impaired driving behaviour.

A more pragmatic approach is taken with controlled medicinal drugs and these appear less often in traffic legislation. Some countries including the UK, have in place a medical defence that allows those suspected of drug-driving to present evidence of the legitimacy of their positive test result. When comparing different drug-driving schemes internationally, it is important that the limits set need to be considered alongside their legal system, which is often different from the UK.

It is becoming increasingly well known that drivers who misuse psychoactive substances may take more than one psychoactive substance together at one time before driving. In many instances this includes the use of alcohol as highlighted in the Technical report ‘Driving under the influence of drugs’. 16

The Panel recommends that some discussion is needed with regard to the approach taken when more than one substance is detected in the evidential sample. Particularly whether consideration should be given to substances with a known impairing effect that are present below the level currently set in legislation but, may in combination with other psychoactive substances be a risk to driver safety. In some countries a limit has been set for a drug class (e.g., the amphetamines), such that an offence occurs if any combination of the different drugs within the class summatively exceed the cut-off.

There is also growing awareness that drugs with similar pharmacological mechanisms of action to those included in the section 5A legislation, but which are not controlled other than through the provisions of the Psychoactive Substances Act 2016, pose similar impairing effects on driving performance. In addition, new evidence is emerging for some drugs controlled under the Misuse of Drugs Act (1971) and these have been included in drug driving legislation elsewhere. Gamma-hydroxybutyrate (GHB) with sedative and anaesthetic effects is a good example. The Panel recommends that the Department for Transport keeps a watchful brief on developments in other drug-driving communities as well as the scientific literature in order to make informed decisions about the addition of further drugs to the section 5A drug-driving legislation.

11 The Capability of UK Forensic Laboratories to Undertake Analyses Using Blood or Oral Fluid

The Forensic Science Regulator has issued Codes of Practice and Conduct for the analysis and reporting of specimens in relation to section 5A of the Road Traffic Act 1988 (Forensic Science Regulator, 2016a). The Panel noted that any laboratory undertaking analysis of blood where the results may be used for a prosecution under section 5A (Road Traffic Act 1988) would need to meet the following requirements:

- Be accredited to ISO 17025 (ISO, 2005);
- The analysis of blood samples for the section 5A offence shall be specifically listed in the scope of accreditation;
- Comply with the Codes of Practice and Conduct for the analysis and reporting of blood specimens (Forensic Science Regulator, 2016b).

It was also noted that the laboratory should comply with the guidance on forensic toxicology issued by the United Kingdom and Ireland Association of Forensic Toxicologists (http://www.ukiaft.co.uk/).

11.1 Current Drug-Driving Collection Procedures

The Panel noted that the Dräger DrugTest® 5000 device (Dräger UK, 2016) was currently being used successfully in police stations as a screening device for OF. The Dräger DrugTest® 5000 device was initially supplied to police stations with 250 cassettes; some were for testing cannabis only and a number of dual cassettes were available to test for both cannabis and cocaine. In most cases the Dräger DrugTest® 5000 has been held in the custody suite and training is required in order to use the device. It is currently unknown how many police officers have been trained to use the Dräger DrugTest® 5000.

In addition DrugWipe-2® (Securetec, 2016), which cost £18 per device were issued to Roads Policing Officers in the UK with expertise in drink and drug-driving arrests. The cut-off for the UK roadside OF screening devices are 30 μg/L for cocaine and BZE and 10 μg/L for THC. The actual specification was for screening devices to give a positive result in 90 % of instances where the target drug concentration is 140 %, and 100 % positive at 175 %.

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17 The term “laboratory” is used in this document to refer to any person, body or organisation (of whatever form) which undertakes analysis of blood where the results may be used in relation to a prosecution under s5A Road Traffic Act 1988.
At least 90 % of results for 60 % of target drug concentration should be reported as negative and 100 % negative at 25 % (Home Office, 2012).

11.2 TOXICOLOGY TESTING FOR SECTION 5A AND SECTION 4 DRUG-DRIVING OFFENCES

For reporting purposes it was established that the only substances authorised to be declared at the roadside for the section 5A offence are cannabis (THC) or cocaine and BZE following a positive OF screening test. For cannabis, only THC is relevant for the section 5A offence, whereas THC-COOH (the non-active metabolite) is included in the testing panel set by the National Forensic Framework Agreement (Table 42)\(^\text{18}\) for section 4 offences. When drugs other than the 17 compounds listed in the section 5A legislation are detected they can be used for section 4 offences providing an arrest for section 4 is evidenced.

Table 42 Lists the drugs that are covered in Section 4 of the Road Traffic Act, 1998.

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Specific compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamines</td>
<td>Amphetamine, methamphetamine, MDMA, MDA</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Cocaine, BZE</td>
</tr>
<tr>
<td>Cannabinoids</td>
<td>THC, THC-COOH, THC-11-oic acid</td>
</tr>
<tr>
<td>Opiates</td>
<td>Morphine, codeine, dihydrocodeine</td>
</tr>
<tr>
<td>Opioids</td>
<td>Methadone, buprenorphine</td>
</tr>
<tr>
<td>Common benzodiazepines</td>
<td>Diazepam, Temazepam</td>
</tr>
<tr>
<td>Misused benzodiazepines</td>
<td>Alprazolam, phenazepam</td>
</tr>
<tr>
<td>Hallucinogens</td>
<td>Ketamine</td>
</tr>
<tr>
<td>Common cathinones</td>
<td>Mephedrine, methylene, naphyrone, butylone, methylenedioxypyrovalerone (MDPV)</td>
</tr>
<tr>
<td>Common Piperazines</td>
<td>Benylpiperazine (BZP), Trifluoromethyl phenylpiperazine (TFMPP), meta-chlorophenyl piperazine (m-CPP)</td>
</tr>
</tbody>
</table>

\(^\text{18}\) National Forensic Framework Agreement – Next Generation Drug-Driving Panel
11.3 Drug-testing performance by police forces

Between March 4th 2014 and May 31st 2016 the number of individuals apprehended and suspected of driving under the influence of drugs was 3820. Of these, the DrugWipe-2® (Securetec, 2016) and the Dräger DrugTest® 5000 (Dräger UK, 2016) were used in 3747 cases at the roadside (176 Dräger DrugTest® 5000 and 108 DrugWipe-2® were carried out in the station). Fifty-one percent of the individuals (n = 1949) were characterised as requiring no-further-action (NFA). Of the screening tests undertaken 2028 were positive (54 %) and led to an arrest and 1718 individuals provided a blood sample for a confirmatory test undertaken at a police station. Out of the total number of blood sampled analysed 94 individuals were charged with a section 4 offence (6 %) and 750 drivers with a section 5A offence (49 %) offence; 81 individuals failed to provide a sample (Table 43).

LSD, Flunitrazepam and clonazepam were not detected in any of the samples analysed, whereas all of the other drugs included in the section 5A legislation were detected. Cannabis was by far the most common substance detected (57 % of the total positive tests). If BZE and cocaine were combined 32 % of blood tests were above the cut-off and this was 29 % of the total positive tests. Of the 1718 positive blood tests submitted for analysis, 1175 were above the section 5A limits.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cut-off in legislation (≥µg/L)</th>
<th>No positive confirmatory tests above limit</th>
<th>No of positive tests</th>
<th>Median conc observed of all positive tests (≥µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC</td>
<td>2</td>
<td>761</td>
<td>1049</td>
<td>3.5</td>
</tr>
<tr>
<td>Cocaine</td>
<td>10</td>
<td>125</td>
<td>225</td>
<td>12</td>
</tr>
<tr>
<td>BZE</td>
<td>50</td>
<td>248</td>
<td>315</td>
<td>200</td>
</tr>
<tr>
<td>Ketamine</td>
<td>10</td>
<td>7</td>
<td>9</td>
<td>80</td>
</tr>
<tr>
<td>6-MAM</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>49</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>46.5</td>
</tr>
<tr>
<td>MDMA</td>
<td>10</td>
<td>14</td>
<td>15</td>
<td>37</td>
</tr>
<tr>
<td>LSD</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>250</td>
<td>4</td>
<td>20</td>
<td>70</td>
</tr>
<tr>
<td>Morphine</td>
<td>80</td>
<td>6</td>
<td>40</td>
<td>19</td>
</tr>
<tr>
<td>Diazepam</td>
<td>550</td>
<td>2</td>
<td>24</td>
<td>156</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>100</td>
<td>0</td>
<td>1</td>
<td>3.4</td>
</tr>
<tr>
<td>Temazepam</td>
<td>1000</td>
<td>0</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>300</td>
<td>0</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Methadone</td>
<td>500</td>
<td>3</td>
<td>14</td>
<td>32</td>
</tr>
</tbody>
</table>
The Panel thought that it was good practice to collate the available evidence from the Section 4 as well as the section 5A offences. It was noted by the Panel that there is, as yet, no specific offence for failing to provide an OF sample for a preliminary drug screening device under section 6D (2) of the road traffic legislation.

11.4 The capacity of UK FSPs to measure an alternative drug driving matrix
The Panel received some information from the Forensic Framework Management Team (FFMT) in the Home Office about the capability of using OF for evidential testing. It was noted that the accreditation of all suppliers is listed on the UKAS website but is subject to change as the number of laboratories accredited increases. There were currently (June 29th 2016) seven suppliers with ISO 17025 accreditation for the analysis of drugs under the section 5A offence. All FSPs have specific aspects of the section 5A offence for which they are accredited on the UKAS website (UKAS, 2016). All offer the full range of toxicology services although some may use sub-contractual arrangements to achieve this including for the section 4 offence.

11.5 Summary
The Panel was informed by the Forensic Marketplace Team that FSPs have some ability to use OF for drug detection purposes and accreditation for OF testing is undertaken by some suppliers (but not for the section 5A offence), as OF is commonly used in work place drug testing. However, if OF were introduced for evidential testing purposes method development, validation and accreditation would need to take place.

The issue of requiring multiple drug testing with multiple cut-off limits in OF should be achievable based on the scientific literature and is widely used by WADA and in clinical settings. It was noted that FSPs would need to consult on this matter and agree this general principles for this approach.
12 RECOMMENDATIONS OF THE EXPERT PANEL

1) That the Home Office could expand the list of type-approved screening tests to include, in addition to THC and cocaine (which also provides a route to the cocaine metabolite BZE), the amphetamine-type drugs (methamphetamine and MDMA) and ketamine to reflect the growing use of these compounds in driving populations.

2) That whole blood continues to be the most appropriate tool for evidential testing where a per se threshold approach is required such as for medicinal controlled drugs.
   a. Where whole blood is used for evidential tests there should be a specification (minimum standard) for the sample collection kit and the blood collection tube that includes details of the amount of preservative and anticoagulant required.
   b. Assay uncertainty should also be established for the confirmatory test method(s).

3) That OF may be used as an alternative to blood when an LLOQ approach is used.
   a. For evidential testing OF cut-off limits published in the ‘Guide to Type Approval Procedures for Preliminary Drug Testing Devices Used for Transport Law Enforcement in Great Britain’ in 2012 could be used for illicit drugs;
   b. If POCT OF devices are to be used for evidential testing, criteria in terms of sensitivity, specificity and accuracy for OF POCT device(s) should be established by using a type-approval process;
   c. If a commercial device is used to collect OF for evidential purposes, then the recovery of the analytes of interest and the overall reliability of the device should be specified incorporating collection volume, imprecision data and uncertainty of measurement to provide the drug concentration in neat fluid to satisfy the criminal justice system;
   d. A specification (minimum standard) for the sample collection kit and the OF collection tube that includes details of the amount of preservative, stabiliser and buffer should be established;
   e. OF samples should be stored in glass tubes, away from fluorescent light and direct sunlight;
   f. OF samples should be refrigerated (ideally 3 – 5 °C) as quickly as possible after collection and transported to the laboratory in a controlled temperature to avoid bacterial contamination and degradation of drugs;
g. OF samples should be frozen (ideally at -20 °C), if not available for immediate analysis.

4) That as an adjunct to medical assessment, hair testing is an appropriate matrix for re-licensing decisions, since hair testing provides a much longer window of detection than either blood, urine or OF and would enable the determination of a history of past exposures to illicit or medicinal controlled substances.

5) That a new approach is required where more than one substance is detected in the evidential sample particularly alcohol, where consideration should be given to substances with a known impairing effect that are present below the level currently set in legislation but may in combination with other psychoactive substances be a risk to driver safety.

6) That the Department for Transport should develop a process to monitor changes in recreational drug use patterns and consider new evidence as it becomes available in the scientific literature in order to make informed decisions about the addition of substances to the drug-driving legislation.

7) That the Department for Transport should also keep under surveillance changes in other jurisdictions for potential improvements in their practice of monitoring and deterring drug and drink driving.
13. **APPENDIX 1 - TERMS OF REFERENCE**

13.1 **AIM**

To form a panel of experts to review alternative biological matrices that could be used as an evidential matrix for the new drug driving offence.

13.2 **OBJECTIVES**

To identify alternative biological matrices and report on the relative advantages and disadvantages of each matrix in terms of the following:

i. Residence and detection time of drugs in the matrix;
ii. Collection methods and devices used for sample collection;
iii. Stability/lability of the drug(s) in the matrix;
iv. Susceptibility of the matrix to contamination or adulteration (including through any routes of passive exposure or in relation to a specific route of drug administration).

To describe the different methodology and techniques available for the detection of the compounds shown in Table 1 in different matrices and note:

i. The maturity of the methods used for analysis in each matrix;
ii. The analytical uncertainty for the compounds shown in Table 1 at the limits specified in law for whole blood;
iii. Current standard procedures in use to address analytical variation;
iv. The capability of UK forensic laboratories to undertake analyses using each matrix;
v. and likely challenges associated with the use of each matrix.

To establish, for each matrix, whether a relationship exists between the concentrations of drugs (as listed in Table 1) in whole blood and in the matrix and note:

i. The variability of this correlation between individuals;
ii. The variability of this relationship for each drug in Table 1 for each different matrix;
iii. If this relationship is altered according to the approach used to set the limits in blood i.e. zero tolerance or *per se* approach;
iv. If it is possible to use the blood drug cut-offs (established in law) to set equivalent cut-offs in a different matrix;
v. Other drug testing arenas where cut-offs in equivalent matrices have been undertaken (attempted or implemented) as a source of evidence;
vi. Different approaches/frameworks (if possible) for the use of an alternative matrix as an evidential medium for the new drug driving offence in UK legislation.
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