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DRUID
Driving under the Influence of Drugs, Alcohol and Medicines

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RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

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1. Introduction

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1.1. General background

DRUID (Driving under the Influence of Drugs, Alcohol and Medicines) aims to combat the scourge of drink-driving and find answers to the question of the use of drugs or medicines that affect people's ability to drive safely. It will bring together the most experienced organisations in Europe to assemble a co-ordinated set of data resources and measures.

Work package 2 of DRUID will analyse the prevalence of alcohol and other psychoactive substances in accidents and in the general driving population, among others by means of roadside surveys and hospital studies on injured drivers from road traffic accidents. Roadside surveys are carried out in the following 13 countries: Belgium, Czech Republic, Denmark, Finland, Hungary, Italy, Lithuania, Norway, Poland, Portugal, Spain, Sweden and The Netherlands. Hospital studies are carried out in the following 8 countries: Belgium, Denmark, Finland, Hungary, Italy, Lithuania, Sweden and The Netherlands. In those countries where both types of studies are carried out, relative risk estimations for the accident involvement while impaired are calculated.

1.2. Task description

One of the tasks in Work package 2 (task 2.1.2) aims at setting up a uniform design for the epidemiological case-control studies. These can only be carried out in countries where the legislation allows random check of the general driving population and where approval can be obtained regarding taking samples from injured drivers admitted to the hospitals.

Before starting epidemiological case-control studies, co-operation with police, hospitals and toxicologists, will be established and devices to be used for sample collection must be agreed upon. Also approval from medical ethics committees and other ethical and legal issues must be guaranteed.

There are large differences between the EU countries in the possibilities of carrying out this kind of research. The design has been chosen in close co-operation with the results from existing projects (e.g. ROSITA and IMMORTAL) and in a way that aims at maximising the similarities of the surveys in participating countries.

1.3. Contents of the working paper

In order to be able to calculate the relative risk and to compare the prevalence as well as the relative risk in the various countries, a uniform study design has been set up for roadside surveys and hospital studies.

Furthermore, uniform guidelines regarding collection of specimen, transport of specimen as well as analysis of specimen have been derived.

Finally, a decision has been made regarding a core list of 23 substances, including alcohol, to be analysed for in all countries.

This working paper includes all decisions taken as part of the uniform design. The following partners have contributed to the various guidelines: DTF, UGent, SWOV, TOI, KTL, UKL-HD and TFA-UNDP.

2. Guidelines for roadside surveys

Terje Assum, TOI, Giampietro Frison, TFA-UNPD, Tove Hels and Inger Marie Bernhoft, DTF, Sjoerd Houwing and René Mathijssen, SWOV

2.1. Background

During the DRUID WP2 meeting at SWOV January 9-10, 2007, a group was appointed to outline how to ensure representativeness of the roadside survey results. The aim of this document is to establish a joint background for planning roadside surveys among participants in WP2. This joint background will increase the generalization of the results and ensure comparability between results from various countries. DRUID WP2 is a unique possibility to have scientifically valid estimates for prevalence of drink-and-drug driving in various European countries.

2.2. Purpose and principles

A major purpose of the DRUID roadside surveys is to compare the prevalence of psychoactive substance use in traffic between countries. This comparison requires national representativeness. The problem is how to achieve this goal with the practical limitations implied by roadside surveys. These limitations vary between the countries, and may cause limited comparability in the results unless utmost care is taken to ensure comparability.

The general hypothesis of DRUID is that drug use increases accident risk. Consequently the surveys should cover drug use among road users who may cause road accidents. Ideally, all active road users, in all regions of each country, on all roads, in all vehicles, at all times of the year, week and day should be represented in the survey, in order to have the road traffic in each country surveyed in a representative way. For practical reasons, however, covering all aspects of road traffic may be difficult. Roadside surveys will usually require co-operation between the police and researchers. The principles of police work are different from those of research, and the police as well as the researchers have practical and economic restraints.

Consequently, deviations from the ideal principle may be necessary. Deviations from general, national representativeness should preferably be agreed within the DRUID consortium to ensure comparability between countries.

This document describes, how representativeness and comparability can be guaranteed within a practically feasible research design.

Recommendations
Deviations from this design by individual research institutes will have to be substantiated, and approved by the Task and WP leader.

2.3. Representativeness factors

Taking a completely random sample of all road users in a country is impossible since it would include random sampling of road users at a random sample of locations and times. A first simplification which is generally applied to roadside surveys into impaired traffic is a limitation with regard to road user type. Usually only car drivers are included, since it is assumed that psychoactive substance use by this road user category has the greatest impact on road safety consequences. Generally a systematic sample of research locations and times is used. Traffic at the selected research locations and times should be representative of traffic on all roads at all times. At the selected research locations, drivers are generally taken at random from moving traffic. It is important that selected drivers in all countries are tested for alcohol and drugs in a uniform way.

Selection of research locations and times

Both traffic exposure and the prevalence of psychoactive substance use may vary considerably by place (region, research area, road type, research location) and time (year, season, day of the week,

time of the day). Therefore, sample fractions by place and time should be proportional to traffic exposure fractions by place and time. Examples of traffic exposure indicators for car drivers are: population, number of cars, number of license holders, distance travelled (vehicle mileage), time spent in traffic, number of trips.

The research locations and times should be systematically selected in such a way that it is plausible that the resulting sample of car drivers is representative of all car drivers in a country, if necessary after weighting. It is recommended that the selection in all participating countries is made in a (more or less) uniform way.

When subpopulations, such as regions or road types, vary considerably, it is advantageous to sample each subpopulation (stratum) independently. Stratification is the process of grouping members of the population into relatively homogeneous subgroups before sampling, thus ensuring the representation of all subpopulations. Then sampling is applied within each stratum (Wikipedia[1]). For the nationwide result to be valid, samples within each stratum must be weighted by a factor relating to the actual proportion of the whole stratum that the sample constitutes.

To have the road traffic surveyed in a way that represents the whole country, certain factors need to be considered.

These factors are:

- Nation - stratified into regions
- Road network stratified into road types
- Time – stratified into year (season), week, day
- Vehicles - foreign versus only domestic vehicles, motorized vs. non-motorized, two-wheelers versus four-wheelers, etc.
- Road users – pedestrians, riders, drivers, professional drivers vs non-professional drivers, genders, age categories, etc.
- Weighting for traffic volumes

The theoretical principle is that the results of the survey should be representative of the country or nation in question. For practical reasons, such as co-operation with the police, long travel distances, very low traffic volumes in sparsely populated areas, or hospital catchment areas in case/control studies, this may not be possible. Ideally, recording efforts should be equal in all regions and all regions equal in size (expressed by population size or mileage driven). If not, the results in the various regions should be multiplied with different weight factors to ensure equal representation of regions.

Recommendations
It will be accepted that a less representative area is chosen. The reason for this choice, however, should be stated and explained. More importantly, the representativeness of the chosen region or area should be assessed, based on e.g. the population percentage of the region. The group recommends weighting the results according to a factor that is available in all participating countries, i.e. population size. Statistical estimates for the whole country should be made.

Weighting of strata (if stratified sampling)

If a stratified statistical sampling procedure is used, the strata should be weighted together in the end by their size according to the stratification variable. If regions are used as strata according to their population, the regions should be weighted according to population when they are added together.

Road network
The theoretical principle is that the survey samples should represent the total road traffic in each country, a requirement which normally means that all road types should be included, i.e. the same road categories as constitute the basis of the national road accident statistics. This may be impossible for practical reasons such as extremely low traffic volumes on certain road categories, high speed limits making the stopping of cars unsafe, too little space in many city or village streets, the reluctance of the police to spend time on low traffic volume roads, etc. On the one hand, collecting a sufficient

[1] http :.wikipedia.org/wiki/Stratified_ sampling

number of samples on such roads is extremely expensive. Moreover, samples from such low-volume roads will have a marginal impact on the national averages, as the national averages will statistically be dominated by high-volume roads. On the other hand, some people claim that drivers under the influence of drugs and alcohol will choose low-volume byroads, because police checks are infrequent there, and consequently it is important to include such roads. The same arguments may apply to small city, town and village streets in addition to the lack of space which may make it difficult to arrange traffic controls there.

After road types have been surveyed, results must be weighted according to the proportion that traffic volume on the different road types make up of the total traffic volume within the region.

Recommendations
It will be accepted that extremely low-volume roads and streets may be excluded from the survey. The kinds of roads included in and excluded from the survey should be stated and substantiated. Preferably estimates should be made for the whole road network.

How to weight for varying traffic volumes?
To have a representative sample of the road users, the samples from each road section should be weighted according to the road traffic volume of the particular road section as well as month, weekday and hour.

Recommendations
Two different ways of weighting for traffic volume will be accepted. Either the road traffic can be counted when the samples are collected, which is the simplest and most reliable, but more expensive method or existing traffic volume data can be used. The method used should be explained in the national report.

Time
Road traffic occurs all year, all week and 24 hours a day. Consequently, the roadside surveys should also cover all the year, all week and all day. Moreover, the extent of alcohol and drug use or driving under the influence of alcohol and drugs may vary considerably around the year, week and day.

Some people claim that alcohol and drug use vary mostly between week days and weekend days and between day time and night time. Consequently, it would be sufficient to cover week days and week nights, as well as weekend days and weekend nights. The cooperation with the police may also require certain limitations as to times of the year, the week and the day. An important point is that at least both week days and week nights as well as weekend days and weekend nights should be sufficiently covered in the survey to make four-ways significant comparisons, see following example.

Example: Prevalence of alcohol and drugs among drivers of motor vehicles. Per cent (fictional figures).

	Weekdays	Weekends	N
Daytime	0. 2	0.4	
Night time	0.8	1.6	
N			

Week and weekend – day and night is defined in the following 8 time intervals of the week to ensure comparability.

Weekdays		Weekend	
1. Monday to Friday	04:00 to 10:00	5. Saturday and Sunday	04:00 to 10:00
2. Monday to Friday	10:00 to 16:00	6. Saturday and Sunday	10:00 to 16:00
3. Monday to Thursday	16:00 to 22:00	7. Friday to Sunday	16:00 to 22:00
4. Monday to Thursday	22:00 to 04:00	8. Friday to Sunday	22:00 to 04:00

Recommendations
It is recommended to include all times of the year, the week and the day in the surveys. However, there may be variation in travel patterns between countries which may substantiate different definitions. Deviations should be stated and substantiated, and preferably estimations as to whole year, week and day should be made.

Vehicles
All vehicles using the roads and streets make up the road traffic that in total creates the road accidents. Even non-motorized vehicles like pedal bicycles are part of this road traffic. However, the motor vehicles pose more threat to other road users than the non-motorized ones. There may be reason to limit the surveys to certain types of vehicles, such as passenger cars and small vans, excluding heavy vehicles, motor cycles, taxis, mopeds and pedal bicycles. There is also the question of foreign versus domestic vehicles. On the one hand, there is no doubt that foreign vehicles contribute to the accident number in each country. Accidents involving foreign vehicles are recorded in the accident statistics of the country where the accident occurs rather than in the country where the vehicles are registered. On the other hand the national authorities may claim that it is impossible to enforce the national Highway Code to foreign vehicles, even though the national Highway Code applies to foreign vehicles. Including foreign vehicles may also pose language problems in the data collection.

Recommendations
It is recommended to include passenger cars (no more than eight passengers) and small vans (up to 3,500 kilos – demands driving license B) in the surveys, including taxis. It is recommended to include both foreign and domestic vehicles. When conducting the survey, the type of vehicle must be recorded. This way, results can be compared between countries for passenger cars and small vans no matter which types of vehicles are included in the various countries.

Road users
All drivers of *passenger cars (no more than eight passengers) and small vans* should be included, professional as well as non-professional drivers, foreign and domestic drivers etc. As mentioned before, type of vehicle must be recorded in order to be able to compare the recommended vehicle types between countries.

Recommendations
Drivers of the above vehicles are recommended for inclusion. Deviations should be stated and substantiated. Men and women, young, middle aged and elderly people as well as different ethnic groups should be represented in the proportion that they are represented in the road user categories to be included in the roadside surveys. Since the vehicles are stopped at random, the sample should be representative according to these factors. However, when pooling the collected data, results can and should be adjusted later to match the composition of the population in question. It is important to instruct the police to stop vehicles according to some random mechanism rather than according to suspicion of alcohol and drug use, e.g. young men in old vehicles. A random mechanism could be stopping the next vehicle or vehicles when the research personnel are ready for new samples or according to the last digits of the license plate number, etc.

2.4. Information to be recorded from the roadside surveys

Information needed on all randomly selected subjects
The following data are needed on all randomly selected subjects in order to be able to identify high-risk groups, roads and times

- Identification number (for sample collection device and recorded data)
- Date (year, season)
- Time and day (8 time intervals of the week)
- Gender
- Age

- Vehicle type
- Road type
- Clinical signs of impairment*
- Self-reported drug use and time of consumption*

Recommendations
This is the recommended information. However we are aware that it may not be possible to collect all information in some countries.

In addition to this, some partners might want to collect the below data in order to estimate the potential bias resulting from non-response and to assess the sensitivity of saliva testing:

Information needed on refusers

Some people will refuse to take part in the survey by refusing to give a sample of blood or saliva. It is extremely important to count the number of refusers. For those road users the following information is needed:

- Identification number
- Date (year, season)
- Time and day (8 time intervals of the week)
- Gender
- App. age
- Vehicle type
- Road type
- The reason of refusal, e.g.: No time
  Other reason…….. ……..

Recommendations
This is the recommended core information.

In addition to this, some partners might want to collect the below data in order to estimate the potential bias resulting from non-response and to assess the sensitivity of saliva testing:

- Clinical signs of impairment*
- Self-reported drug use and time of consumption*

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* If subjects are tested for drug use on a voluntary basis, self-reported drug use (and signs of impairment) might help to estimate the non-response bias. This is especially of importance for the relative risk estimates, based on comparing blood-tested cases and saliva-tested controls. Without additional information, comparing blood and saliva testing results may lead to incredibly high relative risks. It is not very likely that subjects will report drug use, if they didn't use any. So the risk of over-reporting is negligible. On the other hand, some underreporting is to be expected. Therefore, the self-reported drug use will have to be considered as a lower drug use limit.

3. Guidelines for hospital studies

Kristof Pil, Elke Raes, Thomas Van den Neste, Alain Verstraete, UGent

3.1. Purpose and principles of the hospital studies

A major goal of the DRUID project is to define relative risks for drivers to be injured in an accident while under the influence of psychoactive substances. This will be achieved in 8 countries (Belgium, Finland, Denmark, Sweden, the Netherlands, Hungary, Lithuania, Italy) by a case-control study.

Controls are drivers stopped from general traffic at randomly selected sites and times (as described in the guidelines for roadside surveys). Cases are drivers who have been injured in traffic accidents.

Designing a hospital study that is both scientifically correct and practically feasible is hard because

- The design can not interfere with the patient care within the emergency department.
- Other practical constraints within the hospitals have to be addressed.
- Research data have to be comparable with data from task 2.2.a (roadside surveys) to be able to calculate relative risks (task 2.3).

This document intends to provide some guidelines on how to do this.

3.2. Cooperation with roadside surveys

To have the highest statistical power in risk calculation, it is ideal to conduct the roadside surveys in the catchment areas of the hospitals.

However, the goal of the roadside surveys is double:

- To supply the controls for the hospital-cases
- To provide a representative sample of drivers for determination of prevalence of psychoactive substances within the general driving population.

Recommendations to address this ambiguity have been described in Chapter 2 of this document.

3.3. Selection of the study population

The study population is achieved by “multistage sampling”. First a selection of hospitals is made. Secondly, only patients that match a number of well-defined inclusion criteria are selected.

Selection of hospitals

Different criteria have to be considered for the selection of the hospitals.

- Willingness to cooperate.
- Geographical distribution. This is especially important when roadside surveys are organised in the hospital catchment areas in order to increase representativeness of the roadside survey.
- Influx of injured drivers. A small amount of large hospitals can be preferred over a large amount of small hospitals (with better geographical distribution) because the data collection is easier to control.

Once a first selection of hospitals is made, it is recommended to perform pilot tests in each hospital over a limited period of time (e.g. 2 weeks). The number of incoming injured drivers has to be recorded including information concerning the inclusion criteria. These data can be extrapolated to the expected duration of the study to see if the desired sample size can be obtained. If necessary, the number of hospitals can then be adjusted.

Each partner has to check if approval of the ethical committee of the hospitals and informed consent of the patients is necessary.

Hospital personnel

It is advised to have dedicated personnel present in each hospital, e.g. a local supervisor (MD) and a local coordinator (nurse). A researcher should regularly visit all hospitals to check for problems and make changes if necessary.

Each hospital should receive a detailed manual explaining all aspects of the study (e.g. inclusion criteria, data to be gathered and correct interpretation of all forms).

Inclusion criteria

It is necessary to define precise inclusion criteria for the subjects. To increase homogeneity across Europe, it would be preferred that these are the same in all countries. However due to practical and legal issues national differences might be necessary. Therefore a list of inclusion criteria that all countries need to comply with has been made and an additional list of criteria on which countries can decide for themselves.

Recommendations

Deviations from these inclusion criteria have to be substantiated, and approved by the Task and WP leader.

Obligatory inclusion criteria:

- Driver of a motorized vehicle
- Injured in accident on a public road or in the direct vicinity of a public road
- Only primary admissions, not patients transferred from other hospitals
- Admissions because of traumatological reasons
- Time interval between admission and sampling should be less than 3 hours
- Severity of injuries: MAIS (Maximum Abbreviated Injury Scale) 2 or higher

National inclusion criteria (each country decides whether to apply these criteria):

- Inclusion of injured bicycle riders
- Age. It is suggested to include only drivers older than 18, since informed consent is easier to obtain
- Inclusion of drivers killed on the spot. In most countries this is not possible due to practical or legal reasons
- Inclusion of foreign drivers. This is recommended since the study investigates the driving population within a country and not only the native driving population
- Inclusion of professional drivers

Minimum data to be gathered

The data that can be obtained is limited because of the low accessibility of the patients (e.g. short stay in hospital, unconsciousness, deceased in hospital, ...) and practical concerns from the hospital staff.

Therefore it is advised to collect only a limited number of data which have to be standardized across Europe. The minimum data to be gathered can be divided into three groups:

- Toxicological information: the substances and analytical cut-offs have been standardized across Europe
- Patient information:
    - Identification number (for labelling of samples and recorded data)
    - Age
    - Gender

    - Time + date of sampling
    - Medication/Fluids administered prior to blood sampling
    - Severity of injuries: MAIS
- Accident data:
    - Time + date
    - Type of vehicle
    - Single vehicle accident: yes/no
    - Driver's license: yes/yes, but suspended/no
    - Professional use of vehicle: yes/no (only if professional drivers are included)

If possible, more information can be gathered in each country, e.g. the degree of damage to the vehicle.

Collection of patient information

The questioning of the patient should preferably be done face-to-face. If this is impossible (e.g. patient remains unconscious), partial information can be gathered from other sources (e.g. medical record). It should be stressed that the questionnaire is anonymous. Consequently, non-response can be reduced to a minimum.

When patients refuse to cooperate with the study, the reason must be recorded.

Proxy-informants can be allowed to give certain information, if patients are unable to answer.

Protection of patient information

All necessary efforts should be made to guarantee the privacy of the patient and the confidentiality of the doctor-patient relation:

- All information is gathered under supervision of an MD.
- No references about the inclusion of a patient in the study can be made in the medical files of the patient.
- The blood samples gathered for this study are treated in a separate procedure and can never be used for clinical or forensic purposes.
- The toxicological analysis and data processing are done anonymously. All forms and samples are given a unique and anonymous code, of which only the local coordinator has the key. An additional coding is performed in the centre where all data are centralised; the files containing the decoding keys should be password-protected.
- The results of the analysis can not be given out to anyone outside the laboratory and the responsible partner for data processing.

Collection of accident information

Collection of accident information will in some countries require cooperation between the police and researchers. This is not always easy since the principles of police work are different from those of research. Since the terms of the cooperation depend on the local situation, each partner should check with the police how this can be organised. Points of particular interest are reliable collection of data and guaranteeing the anonymity of the patients included in the study.

Time frame

Both traffic accidents and the prevalence of psychoactive substance use may vary by time. Consequently, the roadside surveys should also cover all year, all week and all day. Since the same logic applies to the roadside surveys, the time frame for collection of samples in both studies can be the same.

4. List of core substances

Kristof Pil, Elke Raes, Thomas Van den Neste, Alain Verstraete, UGent

The following list of core substances (to be analysed for in all countries that participate in the roadside survey and/or the hospital study) as well as analytical cut-off values for analyses of both blood and saliva were decided upon based on discussions between all partners. These were carried out by means of email and personal communication and the final decision was made at the WP2 meeting, January 2007.

Besides this core list, each country will add a a minimum of 3 extra substances for analysis, based on knowledge on distribution in the various countries and impairing effect on driving performance (e.g. based on pharmacological profile or previous studies).

Substance	Whole blood analytical cut-off (ng/mL)	Saliva analytical cut-off (ng/mL)
Ethanol *	0,1 g/L	0,1 g/L
Morphine	10	20
Amphetamine	20	25
MDMA	20	25
MDA	20	25
Cocaine	10	10
THC	1	1
THCCOOH	5	NR
Diazepam	20	5
Alprazolam	10	1
Clonazepam	10	1
Benzoylecgonine	50	10
Codeine	10	20
6-acetylmorphine	10	5
Methamphetamine	20	25
Methadone	10	20
Oxazepam	50	5
Nordiazepam	20	1
Zopiclone	10	10
MDEA	20	25
Lorazepam	10	1
Flunitrazepam	2	1
Zolpidem	20	10

* Quantitative breath analyser results valid as well

5. Selection of saliva collection devices

Charlotta Engblom and Anna Rantanen, KTL

As decided at the WP2 meeting in Leidschendam in January 2007 the selection on oral fluid collection device should be made before 1.4.2007. On 16 February 2007, KTL delivered a report to UGent and DTF on testing of various devices (Kaarina Langel, Charlotta Engblom, Anna Rantanen, Teemu Gunnar, Kari Ariniemi and Pirjo Lillsunde: Suitability of Oral Fluid Collection Devices)

After that, some further evaluation on one device has been made by both KTL and UGent. The unanimous suggestion of both KTL and UGent is that the Statsure Saliva Sampler should be used for oral fluid collection in DRUID.

Some results are presented here as rationale for the decision.

The recovery of all drugs tested as ratios against a reference point are shown in the following table:

Collection device	Recovery Descriptive	AM	MDMA	THC	Cocaine	Morphine	Codeine	Diaze-pam	Alpra-zolam	Ethanol
Statsure Saliva Sampler	N	6	6	6	6	6	6	6	6	3
	Minimum	0,837	0,798	0,771	0,785	0,745	0,740	0,780	0,812	0,795
	Maximum	0,956	0,898	0,931	0,907	1,165	0,872	0,945	1,001	0,815
	Mean	0,887	0,863	0,854	0,856	0,885	0,813	0,874	0,911	0,805
	S.E of mean	0,021	0,015	0,024	0,017	0,061	0,019	0,022	0,028	0,006
	Std. Dev.	0,051	0,038	0,060	0,042	0,149	0,046	0,055	0,068	0,010
	Median	0,883	0,881	0,841	0,870	0,842	0,817	0,874	0,916	0,805

The volume of oral fluid collected from six test persons was in average 0.952 ml and collection time was less than two minutes. The device is equipped with a volume adequacy indicator which changes colour when 1 ml is collected. The volume of the buffer used in the device was in average 0.970 ml. The comments from the test persons were mostly positive: easy; fast and simple to use; neutral taste; feels a little unpleasant under the tongue; saliva drops from the pad when taking it off the mouth.

In all, we think that some thoughts have to be given to the regime for quantification of volume of oral fluid from the device but the recovery and stability of the drugs and the easy collection makes the Statsure Saliva Sampler the most suitable device for use in the project.

6. Guidelines for blood and oral fluid specimen collection to test for psychoactive drugs

Kristof Pil, Elke Raes, Thomas Van den Neste, Alain Verstraete, UGent

6.1. Summary

Blood

- 5-10 mL whole blood collection in vacuum tubes containing sodium fluoride and potassium oxalate
- Transportation at 4°C (max. 48 hours)
- Storage in laboratory at -20°C

Oral Fluid

- 1 mL oral fluid collected using StatSure Saliva Sampler.
- Collection according to guidelines by manufacturer
- Transportation at 2-8°C (max. 48 hours)
- Storage in laboratory at -20°C

6.2. Blood samples - Background and further specifications

Blood sample collection

All laboratories analysing blood samples within WP2 will perform analysis on *whole blood*.

Blood is most commonly obtained from the median cubital vein on the anterior forearm. It can be drawn by venipuncture with vacuum tubes (tubes that contain a vacuum that aspirates blood into the tube.).

A tourniquet should be placed on the arm where blood is to be collected. The vein to be used should be palpated to determine its size, depth and direction. The skin should be wiped with a disinfectant swab; an alcohol swab should not be used. The tourniquet can be loosened or removed once blood starts to enter the tube. As agreed at the Leidschendam meeting, a collection tube containing potassium oxalate and sodium fluoride (grey tops) is used. Blood collection tubes should be filled completely to ensure that proper additive concentrations are maintained. The tubes should be gently mixed by inverting 5 to 10 times immediately after collection to prevent coagulation.

All appropriate documents have to be filled in using the same identification number as used to label the collection tubes. Labelling has to be unambiguous.

If more than one container is taken from the same subject, these containers should be identified with the same identification label, but the labels should specify how many containers were drawn from the same subject.

Storage and transportation of blood

Data on the stability of commonly used illicit drugs are scattered over various publications, and a uniform study design has not been applied. Reviews have been provided by Levine and Smith in 1990 (Forensic Science Reviews, 2:147-157) and Skopp and Pötsch in 2002 (Rechtsmedizin, 12:195-202, in German). Since we have to ensure stability of even the most labile compounds (especially cocaine), transportation measures have to be strict. Enzymatic degradation is slowed down by the presence of preservatives in the tubes. Chemical hydrolysis is decreased by low temperatures during transportation. Therefore the blood samples should be transported cooled down or frozen.

Direct transportation of the samples to the laboratory is preferred. If this is impossible due to geographical reasons, samples can be shipped under specific conditions. Before shipping, national

and international regulations should be ascertained. Specimens should be sent as diagnostic specimens and in two containers: the primary container should be wrapped with parafilm or sealing tape around the lid, placed into a plastic bag or a screw cap container with enough absorbent material to absorb all of the fluid in the primary container, and be wrapped by a secondary container such as a cardboard box or mailing tube. This container should prevent crushing of the specimen during transport.

Dry ice should be placed between the plastic bag and the outer shipping container. It should be shipped in insulated outer packaging, and should never be shipped in airtight containers. Useful information and appropriate shipping containers are available from most contractors.

Upon arrival in the laboratory, samples have to be stored at -20°C.

Recommendations
The maximum time for storage at 4°C (=time between sampling at the roadside or at the hospital and freezing) is 2 days.
We acknowledge that these transportation guidelines may not be easy to organize in all countries. This depends on the design of the studies (e.g. the presence of researchers at the roadside, the availability of a mobilhome, geographical situation, presence of dedicated personnel at the hospital, ...)

Therefore it might be a good idea to store samples frozen in the hospitals for e.g. one month and transport them to the laboratory in one shipment. During this transportation insulation and time are important, so that the samples are still frozen when they arrive at the laboratory since the consequences of multiple freeze-thaw cycles for the recovery of drugs in blood samples are unknown. For some drugs transportation at room temperature is not a problem, but some drugs are very unstable drugs (e.g. cocaine and zopiclone). For instance, 97.3% of zopiclone is lost in plasma at 20°C after 3 days (Jourdil N, Mise au point d'une technique de dosage de la zopiclone en LC-MS. Etude de sa stabilité dans les milieux biologiques, Annales de Toxicologie Analytique, vol. XVII, n°3, 2005).

Deviations from these recommendations have to be substantiated, and approved by the Task and WP leader.

6.3. Oral fluid samples - Background and further specifications

Oral fluid sample collection

Oral fluid is collected using the StatSure Saliva Sampler device. Collection has to be done according to the guidelines printed on the instruction leaflet:

1. Do not use device beyond expiration date printed on package (Note: expiration date is minimum 15 months from the order date).
2. Record identification number on tube label.
3. Stand tube upright on flat surface. Check level of buffer fluid; if adequate (see fluid level line), place tube in tube rack. Discard kit if fluid is below fluid line.
4. Remove collector from pouch.
5. Do not rinse mouth. Gather saliva in mouth, do not swallow. Position collector under tongue. Close mouth. Do not chew or suck on pad. Do not move pad around during collection.
6. The collector should remain under the tongue until the indicator turns completely blue. Blue colour indicates collector is saturated with a volume of 1 mL saliva. The collection time is variable and may take 2 to 15 minutes. If the indicator has not turned blue within 15 minutes, the pad should be removed from the mouth and discarded. Recollection with a new device may begin immediately, but only after saliva has first accumulated in the mouth. The collector may be placed in the same position.
7. Open mouth and lift tongue. Remove collector from mouth.
8. Remove cap from transport tube. Insert saturated collector into tube. Do not place collector in mouth after it has been in buffer liquid.

9. Carefully place cap over top of collector stem in tube. Forcefully push cap downward until cap "snaps".
10. Mix saturated collector with buffer by gently shaking tube.

Storage and transportation of oral fluid

According to the guidelines on the instruction leaflet, saliva specimens have to be shipped to the laboratory at 2 to 8°C as soon as possible.

Guidelines on packaging and transportation are the same as for whole blood (see above).

Upon arrival at the laboratory, samples have to be frozen until analysis.

After thawing, a plastic column with white centrepiece bottom and a rubberband (delivered together with every device) is pushed down the collection tube. The saliva:buffer mixture can then easily be recovered and analysed.

Recommendations

UGent has contacted StatSure to determine a time frame in which the devices can be stored safely at 4°C (based on their experience). They stated this time must be determined empirically for each analyte. However in their experience they have never seen degradation of any analyte when it is left out at room temperature for <2 hours or <72 hours if it is kept at 2-8 °C.

Therefore it was decided that the same time frame as used for whole blood transportation (max. 48 hours at cooled temperature) can be used for oral fluid.

Deviations from these recommendations have to be substantiated, and approved by the Task and WP leader.

Contact to StatSure

UGent has informed StatSure on the project DRUID, in total demanding about 45.000 oral fluid devices. A special price was obtained (prices exclude shipping costs):

- $ 1,98 per device
- $ 0,18 per filter (for harvesting the sample)

Contact persons at StatSure are:

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Appendix 1

to Guidelines for roadside survey

Design of DRUID case-control studies

Sjoerd Houwing, SWOV, Inger Marie Bernhoft, DTF, Jacques Commandeur, SWOV, Allan Hansen, DTF, Jari Haukka, KTL, Tove Hels, DTF, René Mathijssen, SWOV

Objective	To determine the relation between psychoactive substance use by car drivers and their risk of being seriously injured in a road accident.
Design	Population-based case-control study.
Subjects	Cases consist of all seriously injured car drivers (MAIS ≥ 2 or equivalent) admitted to the Emergency Department of one or more trauma centres. Controls consist of a systematic sample of the general driving population in the catchment area of the trauma centre(s).
Main outcome measure	Adjusted odds ratios (which approximate relative risk in the case of a rare event, such as a road accident) of getting into an accident when driving under the influence of various psychoactive substances calculated by unconditional logistic regression.
Main explanatory variable	Psychoactive substance use.
Other explanatory variables/confounding factors	Minimum: gender, age, year, season, day of the week and time of day, others may be available, e.g. seat belt use.
Definition of DRUID control sample	Random sample of car drivers drawn from moving traffic on main urban and rural roads in the catchment area of the trauma centre(s). The sample distribution will be stratified according to the distribution of the seriously injured drivers by day of the week and time of the day.
Sampling design for controls	The number of controls in a catchment area will be distributed over time periods according to the national distribution of the seriously injured drivers during the previous three years in order to get approximately the same fraction of controls and cases in every time period.