Centre of Forensic Sciences
Investigators and Submitters

Technical Information Sheets
Toxicology

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Contents

Introduction

The Toxicology Section performs analyses on biological samples (e.g., blood, urine, liver) to determine the absence/presence/concentration(s) of drugs, including alcohol and poisons.

This document is intended as a convenient investigative reference but should not be relied upon as definitive or exhaustive. Please contact the Centre of Forensic Sciences (CFS) Toxicology Section for assistance with questions of an analytical or toxicological nature by e-mail or telephone 647-329-1400 or 647-329-1430. When calling please ask for the appropriate coordinator:

Coroner's Coordinator:

CFSToxicologyCoronerCoordinator@ontario.ca

Criminal Coordinator:

toxcrim@ontario.ca

Examination Strategy and Capability

The screening methods employed in the Toxicology Section are:

1. Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC/MS)
2. Immunoassay (IA)
3. Head-Space GC analysis for volatiles
4. Quadrupole Time-of-Flight MS (QTOF)

The targeted/quantitation methods employed in the Toxicology Section are:

1. GC, GC/MS
2. Liquid Chromatography (LC), LC-MS/MS
3. Head-Space GC analysis for volatiles

Capabilities of screening methods are presented in Appendix 1. While these screening methods have wide-ranging capabilities not all drugs may be reliably detected. Appendix 2 contains a list of compounds that may not be identified by the screening methods but may be detected/quantitated by targeted methods. Many of the compounds contained in this list will not be tested for unless specifically requested. If use of a specific drug is known or suspected and is relevant it should be noted in the case synopsis.

The examination strategy, i.e., determining which tests will be performed in a case, is informed by a variety of sources including case type, case history, nature of submitted samples, analytical protocols and capabilities, and discussions with clients. The initial toxicological analyses conducted for a variety of case types are presented in Appendix 3.

Urgent Cases

Requests for expedited analyses must meet specific criteria before being accepted as an urgent case. This process requires authorization by Toxicology Section management.

Examination

All items are visually examined on receipt to check the seal numbers (if present), the contents, and the integrity of the packaging.

Instrumentation

Chromatography: Gas Chromatography (GC); Liquid Chromatography (LC)
Chromatography is an analytical technique used to separate compounds based on their chemical and structural properties. GC uses a pressurized gas, while LC uses a pressurized liquid, in the separation of compounds.

Immunoassay (IA)
IA detects compounds in biological fluids using a reaction of an antibody or antibodies to its antigen (i.e., the drug). This technique is primarily a screening technique; however, some IA methods are semi-quantitative, e.g., acetaminophen.

Mass Spectrometry (MS)
MS detects, identifies, and quantitates compounds. An MS can be coupled with a GC or an LC.

Quadrupole Time-of-Flight-MS (QTOF)
QTOF detects and identifies compounds. A QTOF is coupled with an LC.

Tandem MS (MS/MS)
MS/MS detects, identifies, and quantitates compounds and is commonly coupled to a GC or LC.

Ultraviolet and Visible (UV/VIS) Spectrophotometry
UV/VIS spectrophotometry identifies and/or quantitates a drug based on its UV and/or visible light-absorbing properties.

Carbon Monoxide
Carbon monoxide is analyzed by visible spectrophotometry. Results are expressed as % carboxyhemoglobin saturation.

Interpretation

Quantitative results may be expressed as 1) a concentration or 2) as < or > a concentration, e.g., when sufficient for interpretation. Blood ethanol interpretations provided in reports are generally limited to cases in which the detected concentration may be associated with fatalities, may be influenced by post-mortem artefacts, may have toxic interactions with other drugs, or in the case of motor vehicle collision, associated with impairment.

Measurement Uncertainty

Measurements made with all scientific instruments are associated with variability. No measurement is exact but is an estimate of the true value. Calculation of measurement uncertainty (MU) employs statistical methods to determine the range of values within which the quantitative result is likely to reside. The MU provides a reasonable estimate of the variability associated with the analytical method and is based on the analysis of matrix-matched quality control samples. A minimum of 10 such analyses are used. The MU is calculated with a confidence of 95.45 per cent using a k-factor based on the degrees of freedom as determined by the Student's *t*-test and the standard deviation of the associated quality control data. The MU is expressed in the same units in which the quantitative result is reported, e.g., ng/mL, mg/L and is reported as: quantitative result $\pm$ MU.

Limitations

The focus of this laboratory is drug toxicity. Clinical blood/urine chemistry analysis, e.g., electrolytes, cell counts, gas saturation, creatinine, is not performed. Analysis for antiepileptic drugs is limited to determining drug

toxicity, when warranted, based on case history. This laboratory does not have validated methods to analyze some sample types, e.g., oral fluid, hair, bile, muscle, brain tissue. There are a variety of analytical issues that may prevent the detection of some of the drugs that this laboratory is commonly capable of detecting, which include:

- matrix effects
    - degree of putrefaction
    - type of sample (e.g., splenic blood)
    - post-mortem interval
    - storage conditions
- volume of sample submitted
- low concentration of the drug/sensitivity of the method

Conversely, some novel, or rarely encountered, drugs not listed in Appendix 1 may be identified by the GC and GC/MS or QTOF screens. In this case, analytical reference material would be acquired (if available) then analysed to confirm identity. There are drugs/compounds for which the CFS Toxicology Section does not have a method, examples of which are provided in Appendix 4.

Glossary

Abbreviations
Analytical results are reported in terms of mg/100 mL, mg/L, or ng/mL, as shown below:

g	gram
mg	milligram
ng	nanogram
L	litre
mL	millilitre

Breakdown Product
A compound produced either inside or outside the body that may or may not be pharmacologically active.

Carboxyhemoglobin saturation
The percentage of hemoglobin bound by carbon monoxide.

Central Nervous System Depression (CNS depression)
A lowering of the functional activity of the brain and/or spinal cord. Depression of the respiratory and the cardio-regulatory centres are most relevant toxicologically.

Confirmation
The process of verifying the presence of a drug by replicate analysis using the same or different analytical technique(s). Confirmation of an immunoassay result is achieved using a more specific analytical technique.

Coroner's Case Analytical Summary
Contains analytical results with the fatal reference and limitations. The Coroner’s Case Analytical Summary is accompanied by an Interpretive Guide with information specific to this report type.

Detected
The drug has been identified in the sample. Identification is based on criteria specific to the analytical technique.

Fatal Reference
A minimum drug concentration at which death has been reliably reported in the forensic literature.

Inconclusive
The presence or absence of a drug could not be determined.

Metabolite
The product of enzymatic conversion of a drug within the body to a different compound that may or may not be pharmacologically active.

No [other] significant findings by a [method name(s)]
This comment is inserted to provide a reference to the methods that were used. Appendices 1 and 5 can be used to identify compounds not listed and that were either not detected or the results were deemed to not be toxicologically significant, e.g., caffeine or nicotine. This may also apply to endogenous compounds, e.g., acetone < 2 mg/100 mL.

Not Detected
The drug is either not present or is present but at an amount that cannot be discerned from other constituents in the sample.

Post-mortem redistribution
A phenomenon that refers to a change (either an increase or a decrease) in blood drug concentration after death; post-mortem redistribution may occur regardless of sampling site but is commonly observed as increased drug concentrations in heart blood as compared to femoral blood.

Putrefaction
The decomposition of organic material that involves micro-organisms.

Report
Contains a comprehensive summary of analytical results accompanied by interpretative conclusions.

Tentative
A drug has been identified by a non-specific screening method (e.g., immunoassay) but has not been confirmed. Further analysis would be required to positively identify the compound.

Therapeutic
The detected drug concentration is generally considered to not be toxicologically significant. The use of this term does not imply clinical efficacy.

Traces
The drug was detected at a concentration below that which can be reliably quantitated. The use of this term does not imply clinical efficacy.

Unconfirmed
A drug has been identified by a screening method but has not been confirmed by a second analysis. Unconfirmed findings may or may not be toxicologically significant.

Appendix 1 – Screening Methods

Drugs that can be reliably detected by screening methods

GC and GC/MS Screen

A

alpha-pyrrolidinovalerophenone (α-PVP)
acetyl fentanyl[2]
amantadine[1]
amitriptyline[2]
amlodipine[2]
amoxapine[2]
amphetamine[2]
amphetamine (4-fluoro)
anabasine
atomoxetine
atropine/hyoscyamine

B

benzocaine
benzofuran (6-(2-aminopropyl, 6-APB)
benztropine[1]
benzylpiperazine (BZP)
bromo-dragonfly
brompheniramine[2]
bupivacaine[1]
bupropion[2]
butylone/ethylone
butyryl fentanyl

C

caffeine[1]
carbamazepine[2]
cathinone (cath)
  n-ethyl-cath
  4-fluorometh-cath
  3-methoxymeth-cath
  4-methyleth-cath
  meth-cath
chlorcyclizine
chlordiazepoxide[2]
chloroquine
chlorpheniramine[2]
chlorpromazine[1]
cisapride[3]
citalopram*[2]
clomipramine[2]
clonidine[1]
clozapine[2]
cocaethylene
cocaine[2]
codeine[2]
cotinine
cyclobenzaprine[2]
cyproheptadine[1]

D

desipramine[2]
dextromethorphan[2]
dextrorphan*
diazepam[2]
diazepam (nor)[2]
dibucaine[3]
dihydrocodeine
diltiazem[2]
diltiazem (desacetyl)[2]
dimethyltryptamine
diphenhydramine[2]
doxepin[2]
doxylamine[2]

E

ephedrine*[2]
estazolam
etizolam[2]
ethylone/butylone

F

x-fluoroamphetamine
fluoxetine[2]
fluoxetine (nor)[2]
flurazepam[2]
flurazepam (n-desalkyl)[2]
fluvoxamine[2]

H

haloperidol[1]
hydrocodone[2]
hydroxychloroquine
hydroxyzine[1]

I

ibogaine
imipramine[2]

K

ketamine[2]

L

lamotrigine[2]
laudanosine
levamisole
lidocaine
loratadine
loxapine[2]

M

maprotiline[1]
meclizine[1]
mefloquine[1]
meperidine[2]
meperidine (nor)[2]
mephedrone[2]
mepivacaine[1]
methadone[2]
methamphetamine[2]
methamphetamine (4-fluoro)
methedrone
methotrimeprazine[2]
methylenedioxyamphetamine (MDA)[2]
methylenedioxyethylamphetamine (MDEA)[2]
methylenedioxymethamphetamine (MDMA)[2]
3,4-methylenedioxypyrovalerone (MDPV)[2]
methylone[2]
methylphenidate[2]
metoclopramide[1]
metoprolol[2]
midazolam[2]
mirtazapine[2]
moclobemide[1]

N

nicotine[1]
nortriptyline[2]

O

olanzapine[2]
orphenadrine[2]
oxybutynin[1]
oxycodone[2]

P

paroxetine[2]
pentadrone
pentazocine[2]
pentoxyphylline[2]
pentylone
phenacetin
phencyclidine (PCP)[2]
phenethylamines (2C-B, 2C-B-Fly, 2C-T-7, PEA)
pheniramine[2]
phenmetrazine
phentermine[1]
piperazine, 1-3 chlorophenyl (mCPP)
piperazine, trifluoromethylphenyl (TFMPP)
p-fluorofentanyl
p-methoxyamphetamine (PMA)[2]
p-methoxymeth-amphetamine (PMMA)
procaine[1]
prochlorperazine[2]
procyclidine[1]
propoxyphene[2]
propranolol[2]
pseudoephedrine*[2]

Q

quetiapine[2]
quinidine[1]

R

ropivacaine

S

scopolamine (hyoscine)[1]
sertraline[2]
strychnine[1]

T

tapentadol
terbinafine
ticlopidine
tramadol[2]
trazodone[2]
trihexphenidyl[2]
trimethoprim[3]
trimipramine[2]
triprolidine[2]

V

valeryl fentanyl
varenicline
venlafaxine[2]
venlafaxine (O-desmethyl)[2]
verapamil[2]

X

xylometazoline

Z

zolpidem[2]
zopiclone breakdown product

QTOF Screen

The QTOF screen is a powerful and sensitive method that can reliably detect the drugs included in the following methods (details are listed in Appendices 5 and 6):

- LC-MS/MS Mix 2
- LC-MS/MS Mix 3 (except carfentanil)
- LC-MS/MS Mix 4
- LC-MS/MS Mix 5 (except: diflunisal, furosemide, ibuprofen, salicylate, vigabatrin)

In addition, the QTOF screen can identify psilocin. The list of drugs potentially identifiable by QTOF is too extensive to list within this document. For questions about a specific drug not listed, please contact the appropriate case coordinator.

*The GC and GC/MS screen and QTOF screen are not capable of distinguishing racemates, therefore compounds such as dextrorphan/levorphanol, citalopram/escitalopram and ephedrine/pseudoephedrine cannot be separated. Similarly, the GC and GC/MS screen cannot distinguish between 2-fluoroamphetamine, 3-fluoroamphetamine, and 4-fluoroamphetamine.

Immunoassay Tests (known cross-reactivity)

Barbiturates:

amobarbital[2]
butalbital[2]
pentobarbital[2]
phenobarbital[2]
secobarbital[2]

Head-space GC-FID analysis for volatiles (screen and quantitation)

acetone
ethanol
isopropanol
methanol
n-propanol (qualitative)

Volatile screen (qualitative only)

difluoroethane
dichloromethane
1,1,1,2-tetrafluoroethane
ethyl acetate
diethyl ether
dimethyl ether
propane
butane
isobutane
toluene
methanol
ethanol
acetone
methyl ethyl ketone
isopropyl alcohol
acetaldehyde
chloroform
gasoline

Appendix 2 – Drugs Requiring Targeted Analysis

Compounds that may not be identified by screening methods but might be detected and/or quantitated by targeted methods.

A

acepromazine[3]
amiloride[3]
antipyrine (phenazone)[1]
atracurium[3]
azacyclonol[3]

B

bromocriptine[3]

C

carbaryl[1]
carbon monoxide[5]
chlorzoxazone[3]
cyanide[2]

D

dantrolene[3]

diflunisal[2]
dipyridamole[3]

E

ethopropazine[3]
ethylene glycol[6]

F

fenfluramine[1]
fenodipine[3]
fenoprofen[3]
formic acid[4]
furosemide[2]

I

ibuprofen[2]
indomethacin[3]

K

ketoconazole[3]

M

mefenamic acid[3]
methaqualone[1]
metronidazole[3]
mexiletine[1]

N

nabumetone[3]

O

oxprenolol[3]

P

pericyazine[3]
phenylbutazone[3]
phenyltoloxamine[1]
physostigmine[1]
pimozide[3]
pindolol[3]
pipotiazine[3]
piroxicam[3]
prazosin[3]

S

sotalol[3]
sufentanil[1]

T

terazosin[3]
terfenadine[3]
tiaprofenate[3]
timolol[3]
tolbutamide[3]
toluene[4]
triamterene[3]

V

valproic acid[4]

vigabatrin[2]

Y

yohimbine[3]

Methods used for the quantitation of compounds identified in the preceding appendices are denoted as follows:

[1] GC-NPD
[2] LC-MS/MS
[3] LC-DAD
[4] GC-FID
[5] Visible spectrophotometry
[6] Qualitative

Appendix 3 – Initial Analyses by Case Type[a]

Alcohol-impaired driving:	Ethanol
Attempted murder:	dependent upon case history
Confirmation of ketoacidosis:	Ethanol (includes acetone), BHB
Death of child < 5 years of age	Ethanol, QTOF Screen, LC-MS/MS Mix 3, IA cannabinoids, IA acetaminophen
Drug-impaired driving:	QTOF Screen, IA cannabinoids, UDM, GHB
Fatal motor vehicle collision (driver) and aviation death:	Ethanol, QTOF Screen, LC-MS/MS Mix 3, IA cannabinoids, CO[b]
Fire-related death[c]:	CO (whole blood required)
Homicide:	Ethanol, QTOF Screen, LC-MS/MS Mix 3, IA cannabinoids
Mandatory inquest:	Ethanol, QTOF Screen, LC-MS/MS Mix 3, IA cannabinoids
Possible drug-related death:	Ethanol, QTOF Screen, LC-MS/MS Mix 3
Rule Out/exclusionary Toxicology:	Ethanol, LC-MS/MS Mix 3
Sexual assault[a]:	dependent upon case history
SIU death investigation:	Ethanol, QTOF Screen, LC-MS/MS Mix 3, IA cannabinoids

[a] dependent upon sample volume
[b] if fire is involved
[c] other analyses may be performed dependent upon evidence/suspicion of intoxication

Appendix 4 – No Method Available

Examples of drugs/compounds for which this laboratory does not have a method

Animal toxins

α-bungarotoxin
conotoxin
maurotoxin
tetrodotoxin

Anesthetic gases

halothane
isoflurane
nitrous oxide

Curare-related toxins

alloferine
toxiferine
tubocurarine

Other

insulin
lead, mercury
lithium
polychlorinated biphenyls (PCB)
succinylcholine
thallium

Appendix 5 – Capability of Quantitative Methods

Barbiturate method (LC-MS/MS)

amobarbital (qualitative)
butalbital
pentobarbital
phenobarbital
phenytoin
primidone
secobarbital

GHB/BHB method (LC-MS/MS)

γ-hydroxybutyrate (GHB)
β-hydroxybutyrate (BHB)

Cannabinoid method (LC-MS/MS)

tetrahydrocannabinol (THC)
THC (11-nor-carboxy; THC-COOH)
THC (11-hydroxy; THC-OH, qualitative)
cannabidiol
cannabinol

Digoxin method (LC-MS/MS)

digoxin
digitoxin (qualitative)

LC-MS/MS Mix 2

benztropine
benzylpiperazine
brompheniramine
caffeine (semi-quantitative)
clonidine
ephedrine
flubromazolam
flualprazolam
haloperidol
ketorolac
loperamide (qualitative)
lidocaine (semi-quantitative)
mitragynine (qualitative)
nicotine (semi-quantitative)
phenylephrine (qualitative)
pseudoephedrine
trimeprazine (qualitative)
warfarin

LC-MS/MS Mix 3

6-monoacetylmorphine (6-MAM; qualitative)
alprazolam
amitriptyline
amphetamine
benzoylecgonine
bupropion
carfentanil
chlorpheniramine
citalopram/escitalopram
clonazepam
clonazepam (7-amino; qualitative)
cocaine
codeine
cyclobenzaprine
dextromethorphan
diazepam
diazepam (nor)
diphenhydramine
fentanyl
flunitrazepam (7-amino)
fluoxetine
fluoxetine (nor)
flurazepam (n-desalkyl)
hydrocodone
hydromorphone
ketamine
ketamine (nor)
lorazepam
meperidine
meperidine (nor)
mephedrone
methadone
methamphetamine
methylenedioxyamphetamine
methylenedioxyethylamphetamine
methylenedioxymethamphetamine
midazolam
mirtazapine
morphine
nortriptyline
olanzapine
oxazepam
oxycodone
oxymorphone
paroxetine
pseudoephedrine
quetiapine
risperidone
sertraline
temazepam
tramadol (cis)
trazodone
venlafaxine
zopiclone

LC-MS/MS Mix 4

acetyl fentanyl
alprazolam (hydroxyl)
amoxapine
bromazepam
buprenorphine
butyryl fentanyl
chlordiazepoxide
chlorpromazine
clobazam
clomipramine
clozapine
demoxepam
desipramine
desomorphine
diltiazem
diltiazem (desacetyl)
doxepin
doxylamine
duloxetine
etizolam
flunitrazepam
flunitrazepam (N-desmethyl)
flurazepam
fluvoxamine
furanyl fentanyl
imipramine
levorphanol/dextrorphan (qualitative)
loxapine
MDPV
methotrimeprazine
methylone
methylphenidate
naloxone
naltrexone
nitrazepam
nitrazepam (7-amino)
orphenadrine
PCP
pentazocine
pheniramine
promethazine
propoxyphene
triazolam
triazolam (hydroxy)
trimipramine
U-47700
venlafaxine (O-desmethyl)
ziprasidone
zolpidem

LC-MS/MS Mix 5

acebutolol
acetaminophen
amiodarone
amlodipine
atenolol
baclofen
carbamazepine (qualitative)
diflunisal
furosemide
gabapentin
guaifenesin
ibuprofen
labetalol
lamotrigine
methocarbamol
metoprolol
naproxen
pregabalin
prochlorperazine
propafenone
propranolol
pseudoephedrine
salicylate
topiramate
verapamil
vigabatrin

Appendix 6 – Capability of Targeted Qualitative Methods

Urine Drug Mix (UDM; LC-MS/MS)

6-monoacetylmorphine (6-MAM)
acetylfentanyl
acetylnorfentanyl
alprazolam
amitriptyline
amlodipine
amoxapine
amphetamine
baclofen
benzoylecgonine
bromazepam
brompheniramine
buprenorphine
buprenorphine glucuronide
bupropion
butyryl fentanyl
carfentanil
chlordiazepoxide
chlorpheniramine
citalopram/escitalopram
clobazam
clomipramine
clonazepam
clonazepam (7-amino)
clozapine
cocaethylene
cocaine
codeine
codeine-6-glucuronide
cyclobenzaprine
demoxepam
desipramine
desomorphine
dextromethorphan
diazepam
diazepam (nor)
diltiazem
diltiazem (desacetyl)
diphenhydramine
doxepin
doxylamine
duloxetine
ephedrine
etizolam
fentanyl
fentanyl (nor)
flualprazolam
flubromazolam
flunitrazepam
flunitrazepam (7-amino)
flunitrazepam (N-desmethyl)
fluoxetine
fluoxetine (nor)
flurazepam
flurazepam (n-desalkyl)
fluvoxamine
furanyl fentanyl
gabapentin
heroin
hydrocodone
hydromorphone
hydromorphone-3-glucuronide
hydroxyalprazolam
hydroxytriazolam
imipramine
ketamine
ketamine (nor)
lamotrigine
levorphanol/dextrorphan
lidocaine
lorazepam
lorazepam glucuronide
loxapine
meperidine
meperidine (nor)
mephedrone
methadone
methamphetamine
methylenedioxyamphetamine
methylenedioxyethylamphetamine
methylenedioxymethamphetamine
methylenedioxypyrovalerone
methylone
methylphenidate

metoprolol
midazolam
mirtazapine
morphine
morphine-3-glucuronide
morphine-6-glucuronide
naloxone
naltrexone
nitrazepam
nitrazepam (7-amino)
nortriptyline
olanzapine
orphenadrine
oxazepam
oxazepam glucuronide
oxycodone
oxymorphone
paroxetine
pentazocine
phenazepam
phencyclidine
pheniramine
pregabalin
propoxyphene
propranolol
pseudoephedrine
quetiapine
risperidone
sertraline
tapentadol
temazepam
temazepam glucuronide
THC-COOH glucuronide
THC-COOH
topiramate
tramadol (cis)
trazodone
triazolam
trimipramine
U-47700
venlafaxine
venlafaxine (O-desmethyl)
zaleplon
ziprasidone
zolpidem
zopiclone