Lifecycle of a NCSCL Toxicology Sample



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November 15, 2023

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1

Presenter's Background

- Wayne Lewallen
 - B.S. Degree in Chemistry
- Worked for the GBI Division of Forensic Sciences: 2001-2011
- Worked at the N.C. State Crime Lab since 2011
 - Over 20 years working Toxicology Cases
- ABFT Certified in Forensic Alcohol Analysis and Forensic Toxicology
- Chemical Analyst permit from the NC DHHS



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Disclaimer

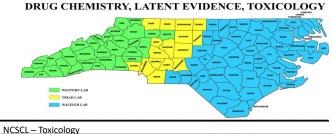
Forensic science is an ever evolving and improving field and the North Carolina State Crime Lab updates its policies and procedures on a regular basis. Understanding that this talk may be viewed at a later date, it is important to note that the information contained here may no longer be valid and if you have questions or concerns about current policies and procedures please contact the North Carolina State Crime Lab.

Finally, analysts cannot speak about cases that are currently being worked or other case specific questions. If you have a case specific question in relation to a case that the crime lab has worked please contact the Lab legal counsel.

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North Carolina State Crime Lab (NCSCL)

- The NC State Crime Laboratory System has labs in:
 - Raleigh (headquarters)
 - Hendersonville (Western Lab)
 - Greensboro (Triad Lab)



Toxicology Status

- Full Toxicology
 - Raleigh: 4 Manager,
 Supervisor, Scientists (1)
 - Triad: 2 –Supervisor, Scientists (1)
 - Western: 8 Supervisor, Scientists (7)
- · Alcohol Toxicology Only
 - Raleigh: 8
 - Triad: 1

- In Training:
 - Raleigh:
 - Alcohol 2
 - Drug 8
 - Triad:
 - Drug 1
- Vacancies
 - Triad: 3
 - Western: 1



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5

What happens when evidence is received at the State Crime Lab

- Evidence is received in the Evidence Control Unit
- A case is started in the Crime Lab's computer database
 - The database is a Laboratory Information Management System (LIMS) called Forensic Advantage (FA)
 - Information supplied by the officer is entered into the FA Web database
 - Each case is given a unique Laboratory Case Number
 - A chain of custody for the evidence is recorded in the FA database
 - The persons handling the evidence are required to enter their personal password to record an entry into the chain of custody
- Toxicology evidence is stored in secure refrigerators



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Toxicologist Training

- Alcohol Toxicology
 - Instrument theory
 - Instrument operation\calibration
 - Pharmacokinetics and Pharmacodynamics
 - Blood Alcohol challenges
 - QA/QC and Measurement Uncertainty
 - Applicable NC General Statutes
 - Competency/Moot Court

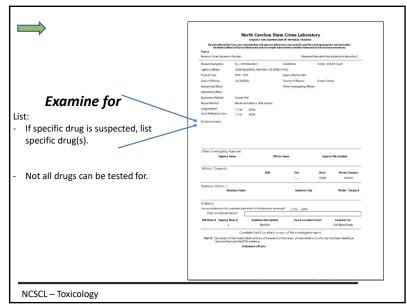
- Drug Toxicology
 - Instrument theory
 - Instrument operation\calibration
 - Extractions
 - Pharmacokinetics and Pharmacodynamics
 - QA/QC and Measurement Uncertainty
 - Applicable NC General Statutes
 - Competency/Moot Court



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Type of Case List: - DWI or - DWI/fatality For cases involving a fatality or serious injury to someone other than the driver, note the fatality of injury in the request for examination. If both alcohol and drug use is suspected, then request "DWI - Blood Alcohol and DWI-Blood Drugs". If Inhalant is suspected request DWI-Inhalant. This will ensure that a full analysis is performed. NCSCL - Toxicology



Toxicology Evidence Submission

- Non-DWI Cases (e.g., drug facilitated assault/rape cases, homicide suspects, custodial neglect):
 - Collect and submit both urine and blood samples in drug facilitated assault/rape cases and other non-DWI type cases
 - Use a leak proof container placed in a zip-lock type plastic bag when submitting a urine sample.
 - The remaining portions of samples will be returned to the submitting agency upon completion of the examination.

11

Toxicology – Evidence Submission

•DWI Cases (Alcohol, Drug, Inhalant): Blood is preferred

- When a negative breath alcohol test result is noted on the Request for Examination of Physical Evidence form or associated case documentation, no blood alcohol analysis will be performed.
- · Record any specific drugs mentioned or found.
- After the analysis is completed, DWI toxicology evidence will be retained by the Laboratory until otherwise authorized.
- Collect 20 mL of blood in two 10 mL blood collection tubes from the subject as soon as possible.
- A minimum of 5 mL of blood is required for the analysis for any other impairing substances.

10

10

Toxicology Evidence Submission

- Alcoholic Beverage Concentrations (e.g., illegally manufactured liquor or other liquids):
 - In cases dealing with multiple buys or numerous samples from the same source, only two random samples will be analyzed.
 - Collect and submit a portion of the suspected Alcoholic Beverage in a sealed vial/container.
 - Submit only a 5-milliliter sample for analysis. The Laboratory will provide small sampling vials if needed.
 - The sample container should not leak air or liquid. Avoid having more air-space than liquid in the container.
 - The remaining portions of samples will be returned to the submitting agency upon completion of the examination.

12

Toxicology – Submission

- •A minimum of 1 mL of blood is required for the analysis for alcohol and/or other volatiles.
- •A minimum of 5 mL of blood is required for the analysis for any other impairing substances.



The condition of the specimen submitted precludes analysis.

13

•Toxicology cannot test cases with insufficient volume or clotted samples

13



Sealed vacutainer



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14

General Misconceptions

"The collection tube was past its expiration date"

• The collection tubes (Vacutainers® - BD)



- The Vacutainer contains a vacuum (negative pressure), designed to draw a blood sample into the tube
- The Vacutainer also may contain additives usually a blood preservative and/or anti-coagulant
 - The color of the rubber stopper on the tube indicates what is added to the tube during its manufacture
- The expiration date applies only to the vacuum in the tube, not the additives
 - When the Vacutainer expires, the seal of the rubber stopper may have failed and allowed air into the Vacutainer
 - The Vacutainer may not draw in all the blood it was designed to

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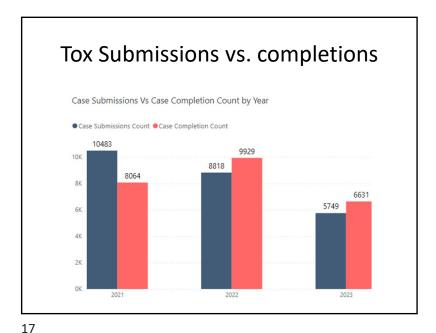
Rush Requests

- The District Attorney's offices can request a "Rush" on their cases via a special web-site or phone call
- The rush requests are evaluated and triaged by a lab manager



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16



Blood Alcohol Concentration (BAC) analysis

- Head-Space Gas Chromatograph (HS-GC) instrument used
- A measured sample of the blood is put in a sealed vial along with a reference standard (internal standard)
- The vial is heated to make the chemicals that evaporate easily, like alcohols, move into the air in the vial (head-space)
- The GC uses a syringe through the rubber seal on the vial, to sample the head-space containing the alcohols
- The head-space sample is analyzed twice by the GC









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Case Work Status

Blood Alcohol

• Blood Drug

- March 2023

- May 2021

Turn around time 30-60 days

Turn around time 60-90

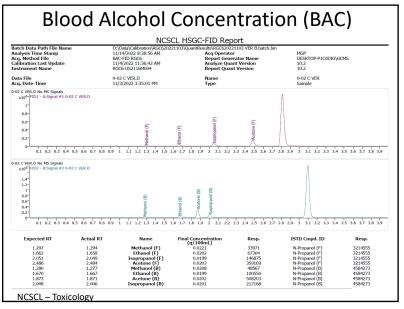
days

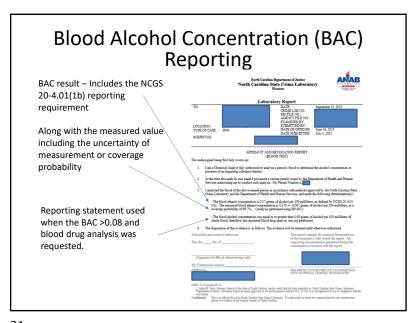
As a result of the current backlog and increased turnaround time of drug toxicology cases - effective February 1,2022, the Toxicology section will suspend testing if an NCGS Schedule 1 substance is identified in DWI cases without a fatality.

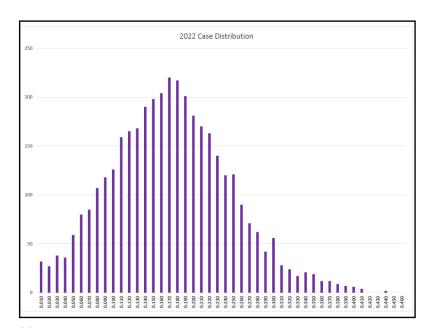


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18







General Misconceptions

"The Lab's BAC test is affected by how the blood sample is collected"
There are different types of tests for alcohol and drugs, and they each have different attributes.

BAC by Immunoassay (used by some hospitals)

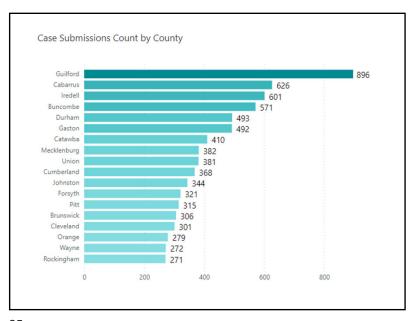
- Uses serum (a processed portion of blood)
 - Causes a BAC result that is higher than when testing whole blood
 - A calculation can be used to convert the SAC to a BAC value
- Damage to red blood cells can affect results, and is caused by...
 - Shaking the blood samples too much
 - Use of a small needle to collect the blood
 - Older samples
- Some cannot tell the difference between different types of alcohol

BAC by HS-GC (used by the NCSCL)

- Uses whole blood
 - As defined by 20.4.01.(1a).a.
- The test is not affected by damaged blood cells
- Identifies the different types of alcohol and other volatiles
 - Ethanol (drinking alcohol)
 - Methanol (wood alcohol)
 - Isopropanol (rubbing alcohol)
 - Acetone (may be elevated in diabetics)
 - 1,1-Difluoroethane (duster spray)
 - Ether

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22



Case Completion Count by County 531 Cabarrus 440 420 402 360 Mecklenburg 346 Forsyth 343 325 315 Orange 313 Cleveland 308 306 Wayne 295

26

28

25

Drug Toxicology Testing Protocol

- Analysis for **drugs in blood** if requested
 - · Screen:
 - 5 panel ELISA
 - QTOF
 - Drugs Confirmation Extractions
 - Confirmatory instruments:
 - Gas Chromatograph / Mass Spectrometer (GC-MS)
 - Liquid Chromatograph / Tandem Mass Spectrometer (LC-MS-MS)
 - Liquid Chromatograph / Quadrupole Time of Flight Mass Spectrometer (QTOF)
- For a compound to make it on our report, it must be identified and confirmed in two separate tests!

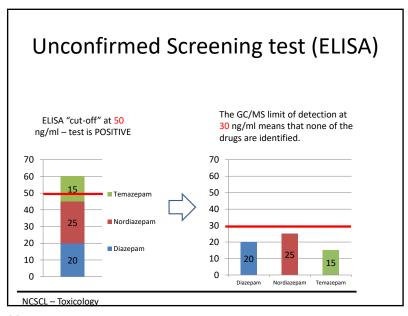
ELISA - Drug Screening

Immunoassay test

Enzyme-linked Immunosorbent assay (ELISA)

Hospitals and work-place drug testing uses this, or a similar test, for drug testing.

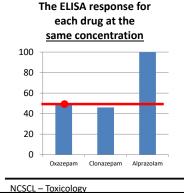
5 Panels
1. Barbiturates
2. Benzodiazepines
3. Cannabinoids
4. Amphetamine/MDA
5. Methamphetamine/MDMA



LC-QTOF-MS

Unconfirmed Screening test (ELISA)

Cross-reactivity



- In a "class" assay, the test has a different sensitivity to each drug - referred to as "Crossreactivity"
- The "cut-off" is set using one drug at a specific concentration
- Therefore, the "cut-off" concentration can not be used to estimate the concentration of a "class" assay drug
- Example: cut-off is set at 50ng/ml with Oxazepam, but 50 ng/ml of Clonazepam will give a negative result

30

Screening on Steroids

- Immunoassay limitations
 - Assay Type
 - Cross Reactivity
 - Expansion requires extensive Lab Validation
- QTOF multiple drugs/metabolites in a single sample.
 - Multiple classes of compounds
 - No Cross Reactivity
 - Additional compounds added with ease
 - Drug Specific Cutoffs



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32

QTOF Panel

Benzodiazepines
7-Aminoclonazepam
- 4 1 0 1
7-Aminoflunitrazepam
Alpha-hydroxyalprazolam
Alprazolam
Bromazepam
Chlordizepoxide
Clonazepam
Delorazepam
Desalkylflurazepam
Diazepam
Diclazepam
Estazolam
Etizolam
Flualprazolam
Flubromazepam
Flubromazolam
Flunitrazepam
Flurazepam
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Midazolam
Nitrazepam
Nordiazepam
Oxazepam
Phenazepam
Triazolam

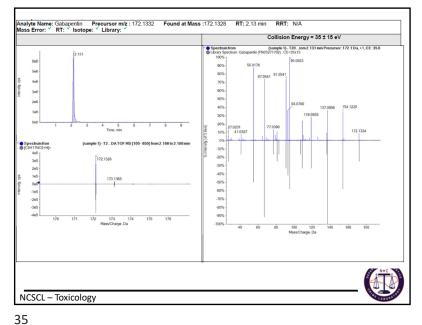
Opiates/Opioids
Buprenorphine
Codeine
Codeine-6beta-D-Glucuronide
Dihydrocodeine
Heroin
Hydrocodone
Hydromorphone
6-Monoacetylmorphine (6MAM)
Morphine
Morphine-3-beta-d-Glucuronide
Norhydrocodone
Noroxycodone
Oxycodone
Oxymorphone -3-beta-D-Glucuronide
Oxymorphone
•

Benzoylecgonine Cocaine Ecgonine Methyl Ester

<u>Fentanyls</u>
Acetyl Fentanyl
Acryl Fentanyl
Carfentanil
Cyclobutyl Fentanyl
Cyclohexyl Fentnanyl
Cyclopropyl Fentanyl
Isobutryl Fentanyl
Meta-Fluorobutyryl Fentanyl
Meta-Fluoroisobutyryl Fentanyl
Methoxyacetyl Fentanyl
Norfentanyl
Ocfentanil
Ortho-Fluorobutyryl Fentanyl
Ortho-Fluoroisobutyryl Fentanyl
Para-Fluorobutyryl Fentanyl
Para-Fluoroisobutyryl Fentany
Para-MethoxyButyryl Fentany
Phenyl Fentanyl
Tetrahydrofuran Fentanyl
Valerylfentanyl
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EDDP	Presecription DP Lidocaine				
	Loratadine				
Methadone					
Carisoprodol	Meclizine				
Meprobamate	Meperidine				
Tramadol	Metaxalone				
O-Desmethyltramadol	Methylphenidate				
Zolpidem (Ambien)	Metoclopramide				
Amitriptyline	Mirtazapine				
Aripiprazole	Olanzapine				
Bupropion	Oxcarbazepine				
Carbamazepine	Paroxetine				
Chlorpheniramine	Pentazocine				
Citalopram	Phenytoin				
Clozapine	Pregablin				
(Dextro)Methorphan	Primidone				
Diphenhydramine	Promethazine				
Doxylamine	Quetiapine				
Duloxetine	Risperidone				
Fluoxetine	Sertraline				
Gabapentin	Tapentadol				
Guaifenesin	Thioridazine				
Haloperidol	Trazodone				
Lamotrigine	Venlafaxine				
Levetiracetam	Verapamil				

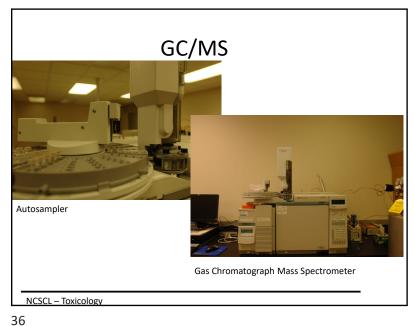
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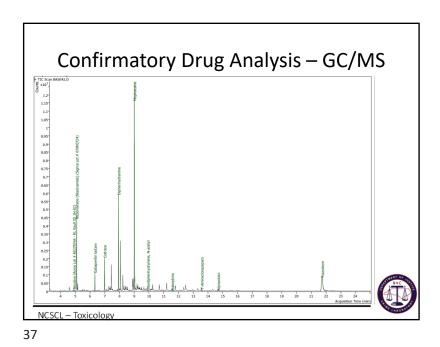


QTOF - Screen

Analyte Peak Name	Peak Height	Area	RT	Mass Error (ppm)	Mass Error	RT	Isotope	Library
7-Aminoclonazepam	312019.80	880600.00	4.16	0.90				
Alpha-Hydroxyalprazolam	4633.80	13420.00	5.87	4.60		V	V	
Alprazolam	136564.00	389200.00	6.07	2.10	<u> </u>	V	V	
Clonazepam	200551.90	545200.00	5.80	1.60	V	V	V	V
Cotinine	261653.60	1012000.00	2.87	-0.70	V	A	V	V
Diphenhydramine	176218.80	513700.00	4.94	1.70	V	V	V	V
Gabapentin	6996147.70	29350000.00	2.13	-2.60	V	V	V	V
Mepivacaine IS	217993.80	693400.00	3.31	0.50	V	V	V	V
Meprobamate	554719.20	1946000.00	4.29	0.40	V	V	V	V
Nalorphine	24900.50	80890.00	2.50	1.60	√	V	V	V
THC-COOH	160268.70	413800.00	7.21	2.00	v	V	V	V
Trazodone	1663173.10	6379000.00	5.04	0.10	>	V	V	V

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Confirmatory Drug Analysis

Confirmatory Drug Analysis – LC/MS/MS

-Ultra Performance Liquid Chromatograph Tandem Mass Spectrometer

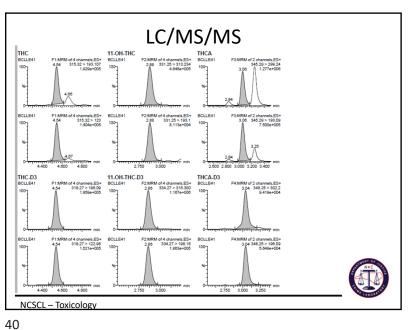
- Used for the identification and quantification of Blood Cannabinoids (THC, 11-OH-THC, and THCA)

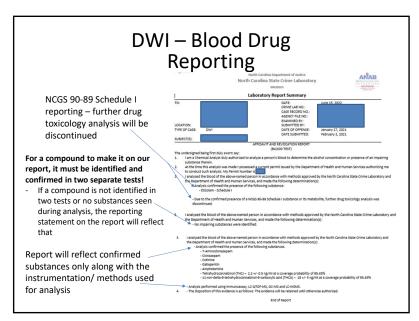


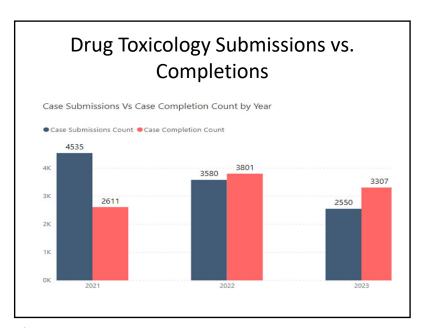
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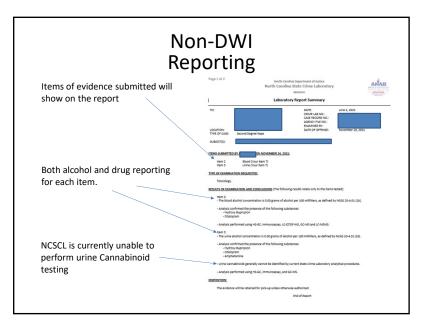
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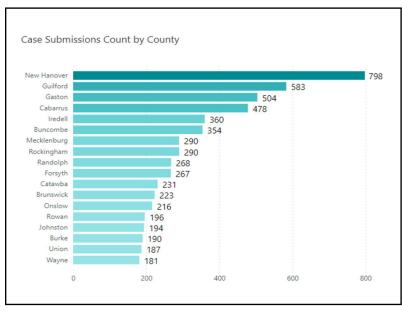
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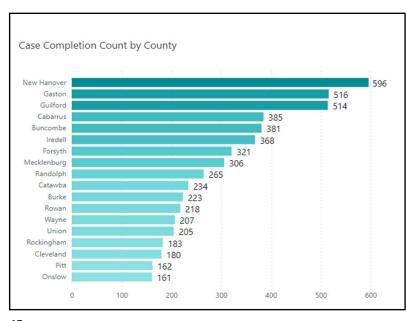


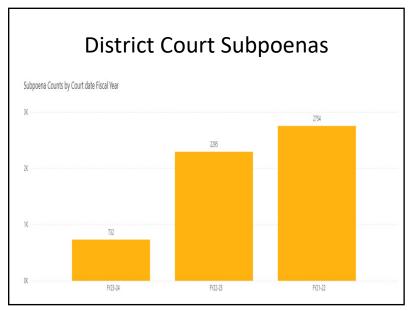


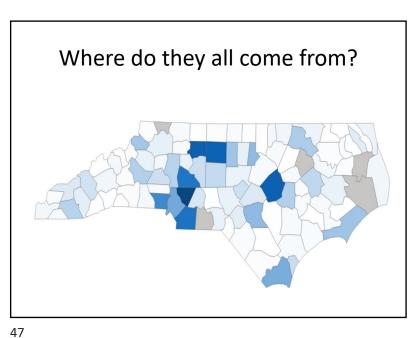


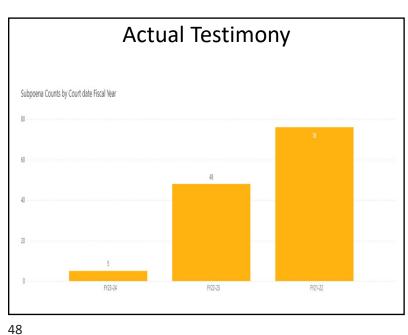


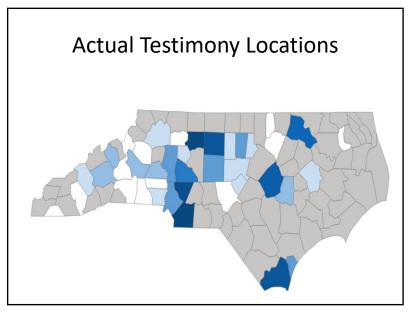


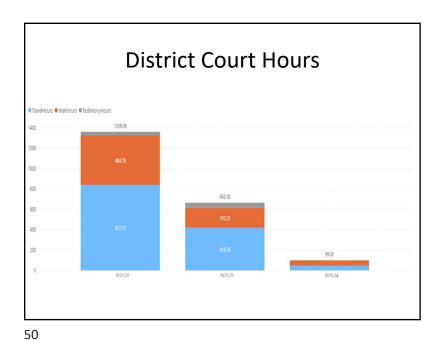


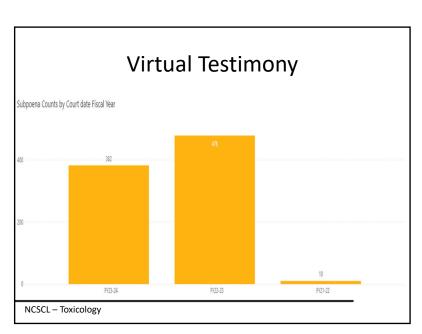




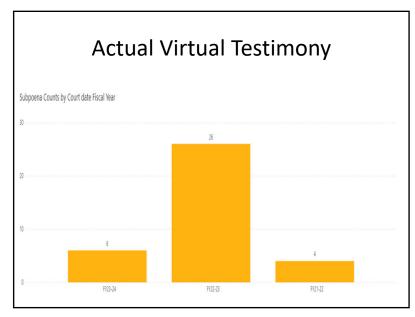


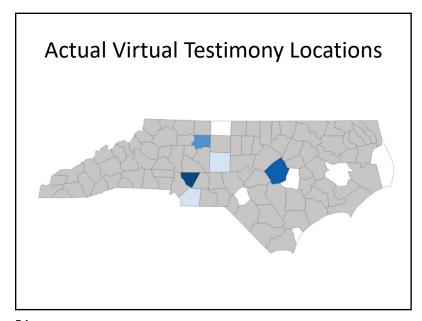


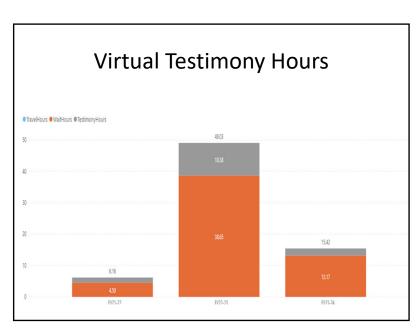


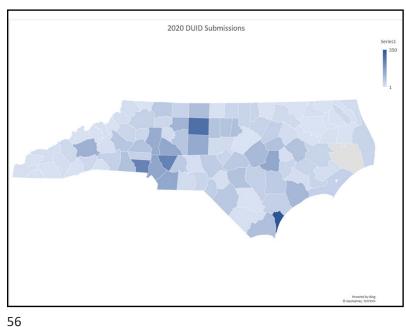


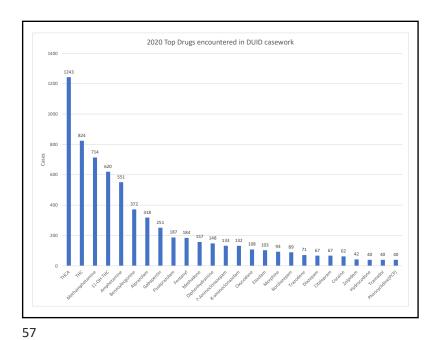
Where are they all coming from?

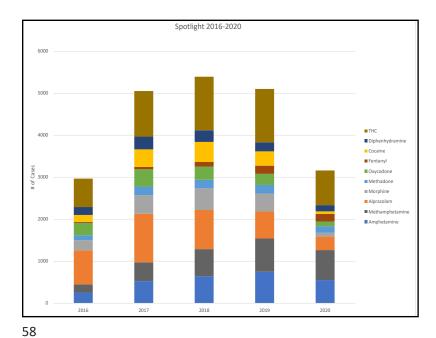












Phenethylamines

900

800

700

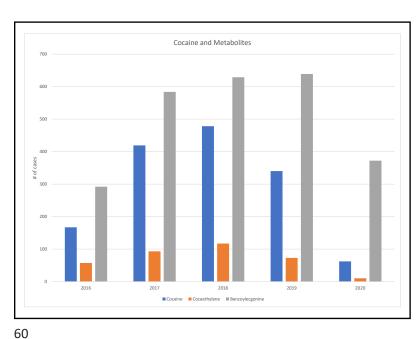
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800

300

200

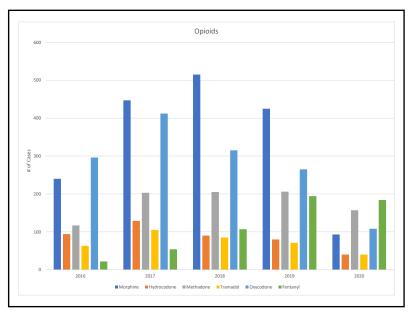
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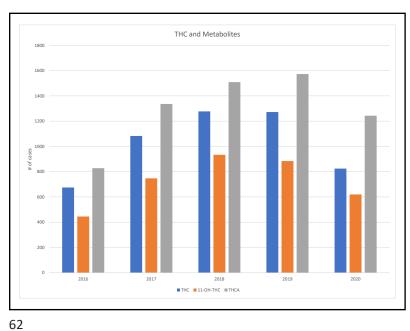


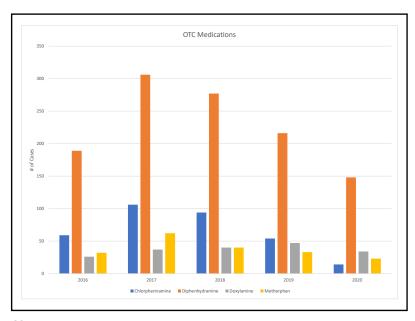
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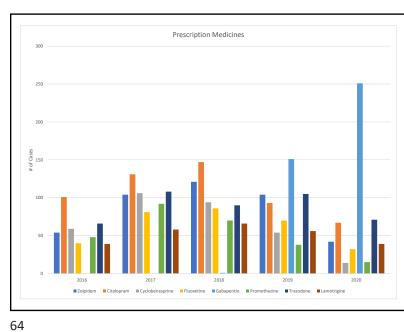
2017 2018

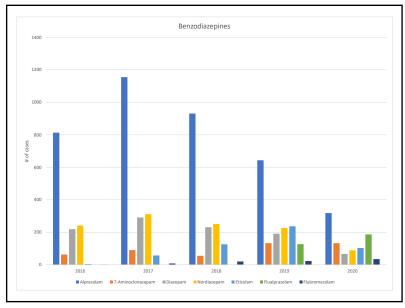
MAmphetamine MDA Methamphetamine MDMA

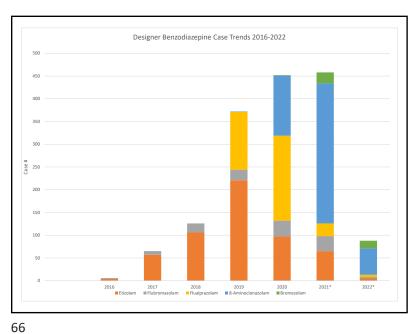


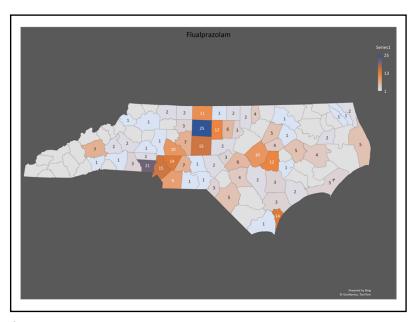


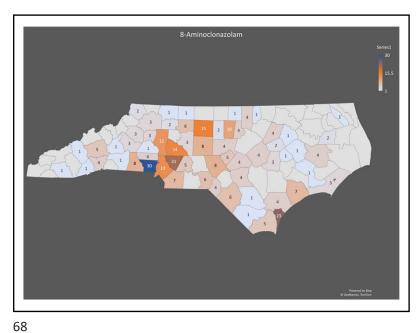


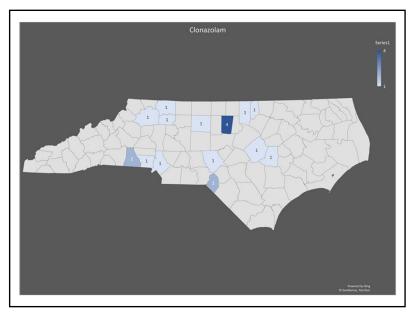












Can the Toxicology Lab determine if a person was impaired?... No

What can the Lab's Forensic Toxicologist testify to about impairment?

- We can identify drugs that are in the blood
- We can tell if the drugs have the <u>potential</u> to be impairing, and generally how they impair
- We can give ranges of how long some drugs can be detected in blood, with an average use



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Can the Toxicology Lab determine if a person was impaired?

No

- Impairment is affected by several factors that are unknown, including:
 - How much drug was used
 - When was it used
 - · What is the persons tolerance to the drug
 - How long have they used it
 - How much do they regularly use
 - Combined effects with other drugs
 - There may be drug present that the lab cannot test for
 - Daily/hourly variations in the person's health



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70

ASB Best Practices

- American Standards Board in conjunction with Organization of Scientific Area Committees (OSAC)
- 9 Tox Standards
 - Measurement Traceability/Validation
 - Scope of Testing DUI/DFSA/Medico-legal
 - Report Content
 - Guidelines for Opinions and Testimony in FT.



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Guidelines for Opinions and Testimony in Forensic Toxicology

Appropriate Opinions and Testimony by a Toxicologist

- Discuss lab report and supporting analytical work
- With appropriate references qualify a concentration as sub-therapeutic, therapeutic, toxic, lethal
- Address Pharmacokinetics\Dynamics of drugs
- Address impairment for the average individual to the extent that effects are consistent with documented pharmacodynamic and toxicodynamic properties of the substance and within the context of a given case

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73

Final Words

- The Toxicology Lab is continually looking to improve our service to the judicial system.
- The processes discussed in this presentation will change over time to improve our product.



Guidelines for Opinions and Testimony in Forensic Toxicology (cont.)

<u>Inappropriate Opinions and Testimony by a Toxicologist</u>

- should not opine as to a specific individual's degree of impairment based solely on a quantitative result.
- should not opine as to the absolute cause of an accident.
- should not opine as to the effects of a drug or combination of drugs on a specific individual without context of a given case. This does not preclude a toxicologist from addressing general effects of drugs at varying concentrations.

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74

Contact Information



Raleigh Laboratory – Toxicology FSM Wayne Lewallen (919) 582-8805 (direct) wlewallen@ncdoj.gov

Triad Regional Laboratory
FSM Amanda Venable(336) 315-4900
avenable@ncdoj.gov

Western Regional Laboratory FSM Ana Baxter (828) 654-0525 <u>abaxter@ncdoj.gov</u>

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75