

Pharmacology, Toxicology and Analysis of:

Gamma-hydroxybutyric acid – GHB

Gamma-butyrolactone - GBL

1,4 Butanediol - 1,4 BD

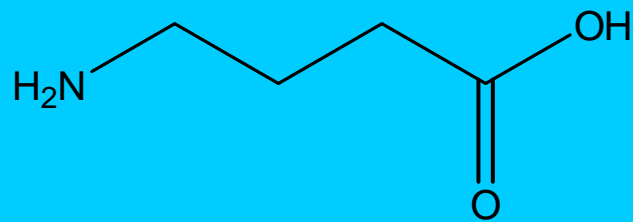
Laureen J. Marinetti, M.S., Ph.D.

California Association of Toxicologists - 2003

What is GHB

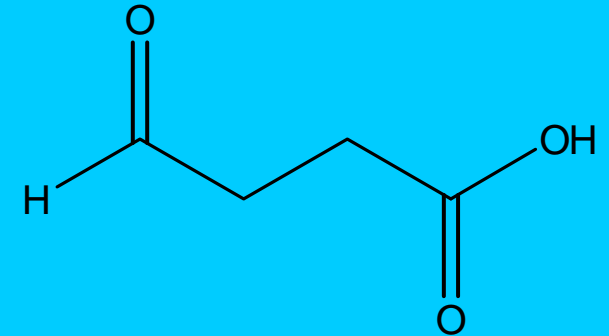
- Central nervous system depressant (CNS) and hypnotic – dose dependent
- Endogenous at very low concentrations in *ante-mortem* mammalian tissue (0.2 - 4.6 mcg/g) ante-mortem human blood (less than 2 mcg/mL) and human urine (less than 10mcg/mL)
- Naturally occurring hydroxy acid metabolite of Gamma-aminobutyric acid (GABA)
- Has a variety of research and medical applications as well as abuse potential
- Over produced in a disease called succinic semi-aldehyde dehydrogenase deficiency (Gibson et al. 1990)

Formation of GHB from GABA

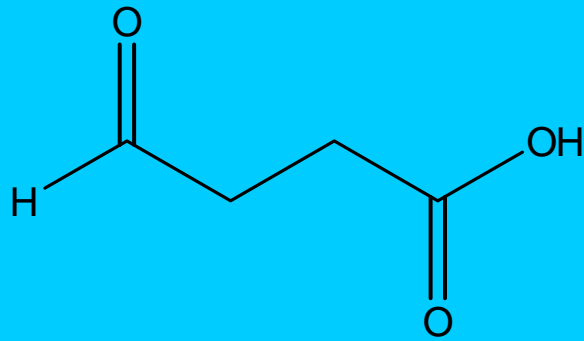


GABA

GABA transaminase

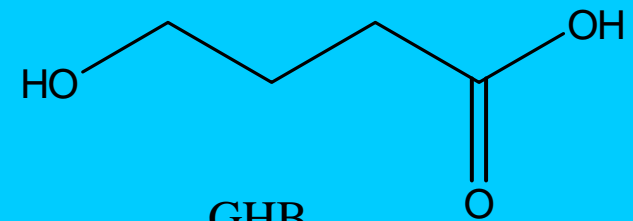


succinic semialdehyde



succinic semialdehyde

SSA reductase



GHB

GHB Time Line

- Synthesized in 1960 by Laborit et al. as a potential GABA analogue - to treat seizure disorder
- Anesthetic properties discovered (Blumenfeld et al. 1962, Helrich et al. 1964)
- No analgesic properties
- Unpredictable duration of action for anesthetic use and random clonic jerking of muscles (Metcalf et al. 1965)
- Growth hormone (GH) releasing property Takahara et al. 1977
- Early 1990's GH release stimulator - banned by FDA
- 1995 to present - Rave parties & illicit drug scene
- 2000 Controlled Substance - Federal Schedule I
- 2002 Xyrem approved - cataplexy treatment - Controlled Substance - Federal Schedule III

Xyrem[®] (Sodium Oxybate) oral solution



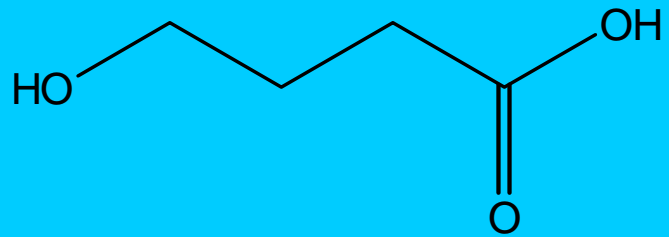
- Formulation
 - Sodium oxybate 500 mg/mL
 - Malic acid 1.3 mg/mL
 - pH 7.5 in purified water USP
- Package Components
 - Childproof cap
 - Press In Bottle Adapter (PIBA)
 - Syringe for measuring each dose

Some Clinical Uses of GHB

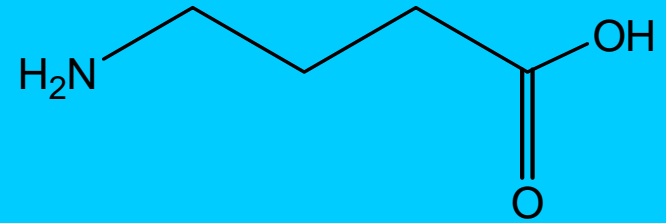
- Anaesthetic induction agent - Etholzner et al. 1995, Detsch et al. 1999
- Narcolepsy/Cataplexy treatment – Mamelak et al. 1977, Scrima et al. 1989, Borgen et al. 1999-2002
- Alcohol withdrawal syndrome – Addolorato et al. 1996, Nimmerrichter et al. 2002
- Protective effect under ischemia/reperfusion injury - Boyd et al. 1992, Ueki 1992

Pharmacology of GHB

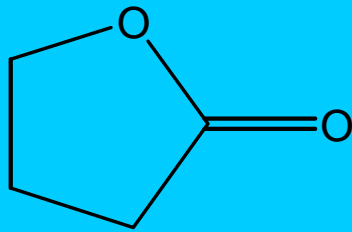
- Alters dopaminergic activity - dose dependent (Roth et al. 1980)
- sub-anesthetic - excite DA neurons (Diana et al. 1991)
- anesthetic - block impulse flow DA neurons = build-up of DA in nerve terminal (Roth 1975, 1976)
- Evidence to suggest same effect on anticholinergic activity (Sethy et al. 1976)
- Active at the GABA(b) receptor (weak agonist) and a receptor of its own (Mathivet et al. 1966)
- Pharmacologically active form GBL and 1,4 BD



GHB



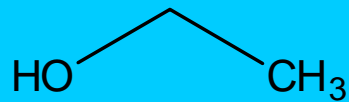
GABA



GBL



1,4 BD



Ethanol

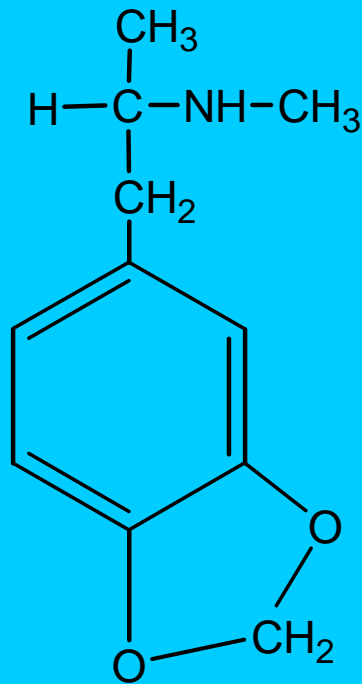
Effects of GHB, GBL & 1,4 BD

- Rapid onset, (~15 - 30 min.) drowsiness, dizziness, visual disturbances, nausea, vomiting, confusion, inebriation, stupor, hypothermia, lowered BP, lowered heart rate, lowered muscle tone, random clonic movements, unconsciousness, severe respiratory depression, loss of bowel and bladder control, coma, death.
- Effects usually last 3-4 hours but this is dose dependent
- Absorbs faster on an empty stomach (Borgen et al. 2000 - Orphan Medical)

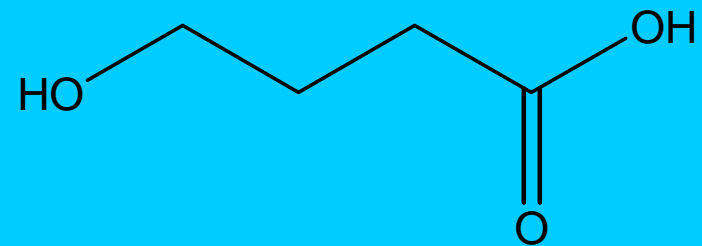
GHB - names

- Gamma OH
- Sodium oxybate
- Natural Sleep 500
- Oxy-Sleep
- GHBA
- G-caps
- G
- Soap
- Liquid X
- *Liquid ecstasy*
- Easy lay
- Salt water
- Vita G
- Georgia home boy
- Grevious bodily harm
- Great Hormones at Bedtime
- Scoops

XTC (MDMA) and liquid XTC (GHB)



Methylenedioxyamphetamine - MDMA



GHB

Growth Hormone Release

- Does increase GH levels
- Correlated with enhancement of slow wave sleep
- GH release does not occur prior to sleep onset (Van Cauter et al.1997)
- Research on alcohol withdrawal syndrome patients - chronic GHB treatment did not see an increase in GH levels (Addolorato et al.1999)
- Later research on these same patients showed that the capacity for GH release with GHB returned after time with abstinence of ethanol

Seizures

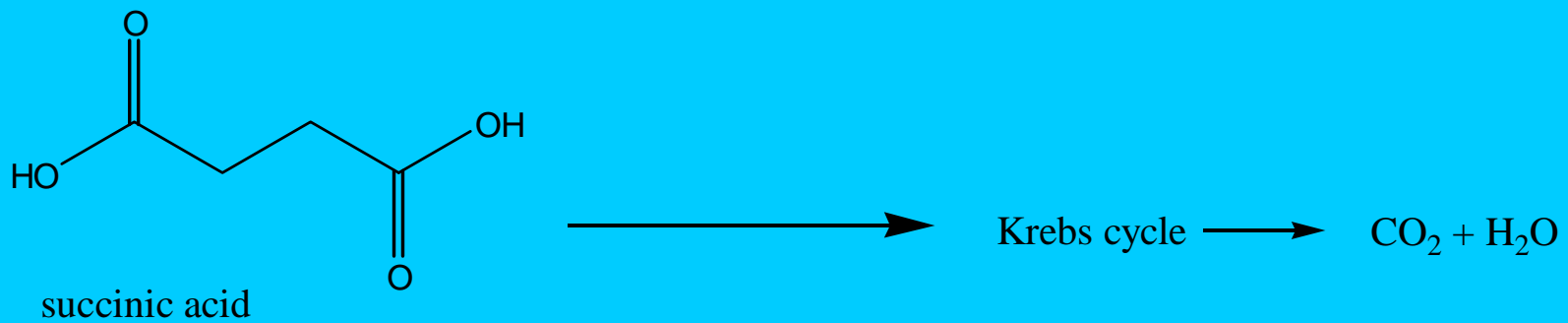
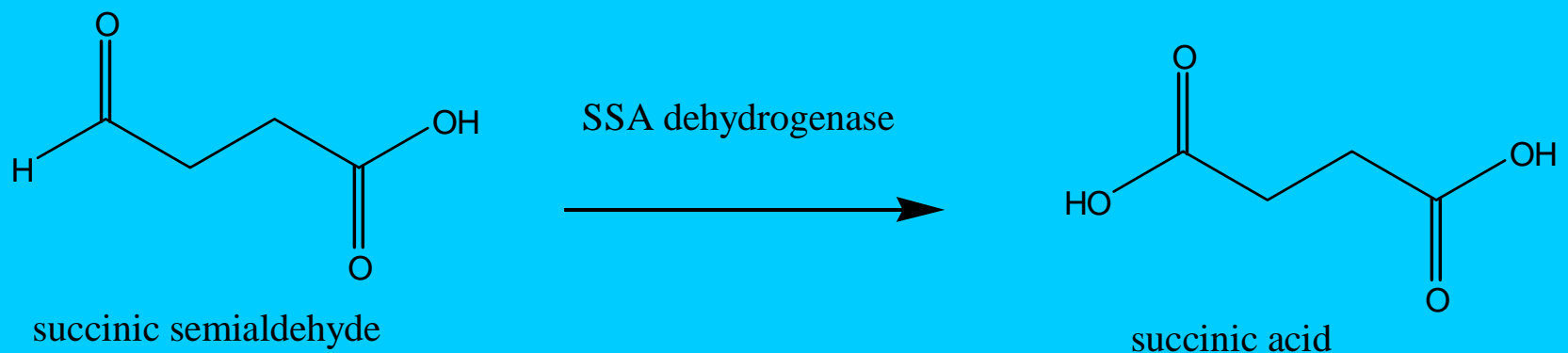
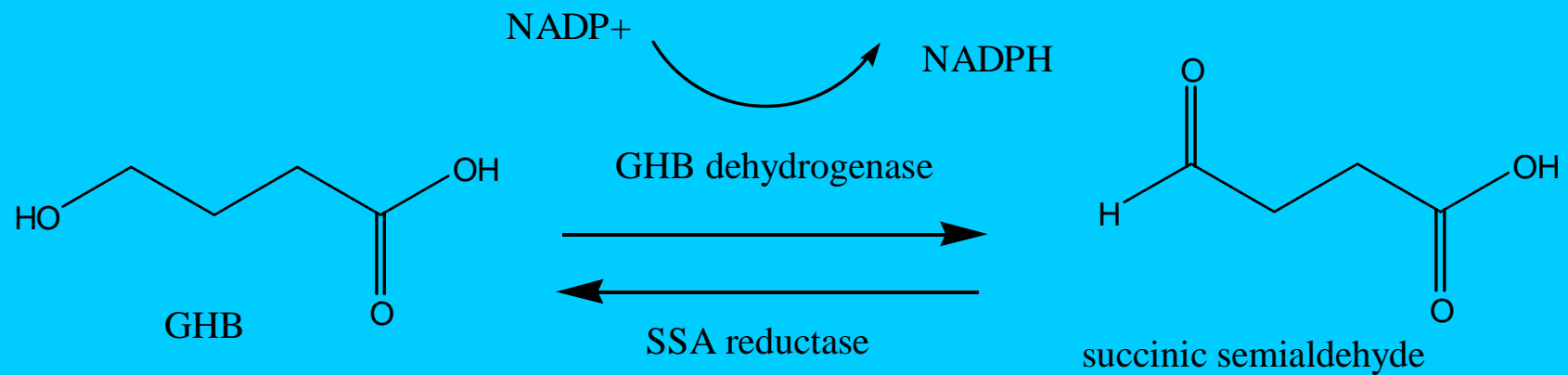
- EEG measurements of humans showed no seizure activity
- Only GHB levels consistent with safe anesthesia have been evaluated (Entholzner et al.1995)
- Does produce random clonic muscle movements (twitching)
- Clonic muscle movements and severe parasympathomimetic activity documented in dogs (toxic and lethal doses) with profuse salivation, urination and defecation (Lund et al.1965)
- Seizures have been documented in some species of animals - mice, rats and cats (Hu et al. 2000, 2001 and Snead et al. 1976)
- why GHB is sometimes referred to as a CNS stimulant

Amnesia ??

- Anecdotal reports of amnestic actions - however...
- There is no *documented amnestic action*
- Low dose GHB did not cause memory impairment in rats (Marinetti and Commissaris, SOFT 2000)
- Not like classic memory impairing drug - scopolamine or the benzodiazepine/ethanol combo - i.e. - Rohypnol in beer or wine
- More likely - GHB causes unconscious with rapid shifts occurring between unconscious and conscious states - this is not amnesia

Metabolism of GHB

- Enzymatic
- End products - CO₂ and H₂O
- No active metabolites
- Zero order kinetics
- No true T-1/2 range = 30min to 2 hours or more dose dependent
- No protein binding
- Does not accumulate nor is it sequestered in fat
- First pass metabolism - 65% oral bioavailability
- Absorbs faster on an empty stomach (Borgen et al.)
- Capacity limited absorption (Palatini et al. 1993)



GHB Dehydrogenase

- Found in brain, liver, heart, spleen, testis, brown fat and kidney
- Drug inhibitors include; valproate, ethosuximide, salicylate, amobarbital, phenytoin, disulfiram and cyanide
- Not known if therapeutic levels of the above mentioned drugs in humans would significantly inhibit GHB metabolism if taken concurrently with GHB

GHB dehydrogenase inhibitors

- Natural metabolic products of disease states;
- maple sugar urine disease products including alpha keto isovalerate, alpha keto isocaproate, alpha keto beta-methyl n-valerate
- PKU or phenylketonuria product - phenylacetate
- Untreated diabetes or starvation state products - ketone bodies including D-beta-hydroxybutyrate and acetoacetate (Kaufman and Nelson 1987)

What is gamma butyrolactone (GBL)?

- An industrial chemical - ingredient in cleaners, solvents, paint removers, engine degreasers, nail polish removers, etc...
- Found in food - wine at ~ 5mcg/mL (Vose et al. 2001) ~0.004g GBL per 750mL bottle of wine
- The pro-drug of GHB
- It is pharmacologically inactive (Roth et al. 1966)
- The pre-cursor of GHB in illicit manufacture
- Oily liquid - many chemical synonyms
- List I Chemical under Controlled Substances Act

Chemical Synonyms for GBL

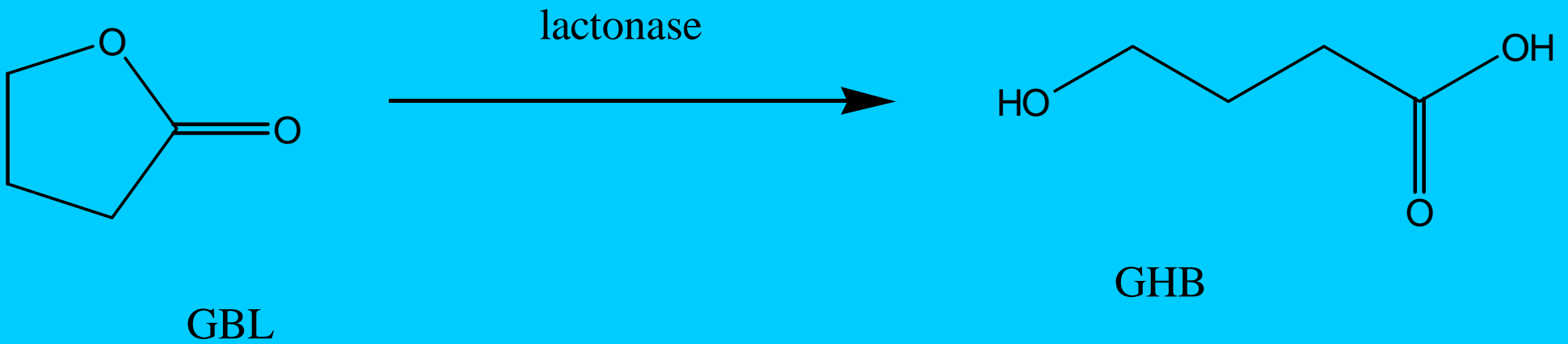
dihydro-2(3H) furanone	4-hydroxy-gamma-lactone	4-deoxytetrionic acid
butyrolactone-4	butyrylactone	1,2-butanolide
4-butyrolactone	butyrl lactone	1,4-butanolide
butyric acid	gamma-6480	4-hydroxybutyric acid-gamma lactone
butyric acid lactone	gamma BL	gamma hydroxybutyric acid cyclic ester
butyrolactone	BLO or BLON	NCI-C55875
alpha-butyrolactone	4-butanolide	tetrahydro-2-furanone

Effects of GBL

- Same as GHB
- GBL is rapidly converted to GHB in vivo by the enzyme lactonase
- Onset of action faster than GHB because GBL is absorbed faster (more lipophilic)

Metabolism of GBL

In vivo: blood and liver



Lactonase Enzyme

- Found in liver and blood
- No lactonase activity found in brain, kidney, heart, lung, skeletal muscle, intestine, urine, or CSF (Roth and Giarman 1966)
- Calcium dependent enzyme - not inhibited by NaF it is inhibited by Na EDTA (Fishbein and Bessman 1966)

What is 1,4 Butanediol (1,4BD)?

- Industrial compound
- Not used to illicitly manufacture GHB - industrial process and cannot be accomplished in a clandestine lab
- Ingredient in: SomatoPro, Revitalize Plus, Serenity, Enliven, GHRE, NRG3, Thunder Nectar, Weight Belt Cleaner, Pine Needle Oil or Extract
- Illegal to distribute for human consumption under CSA as a GHB analog - depends on jurisdiction !?

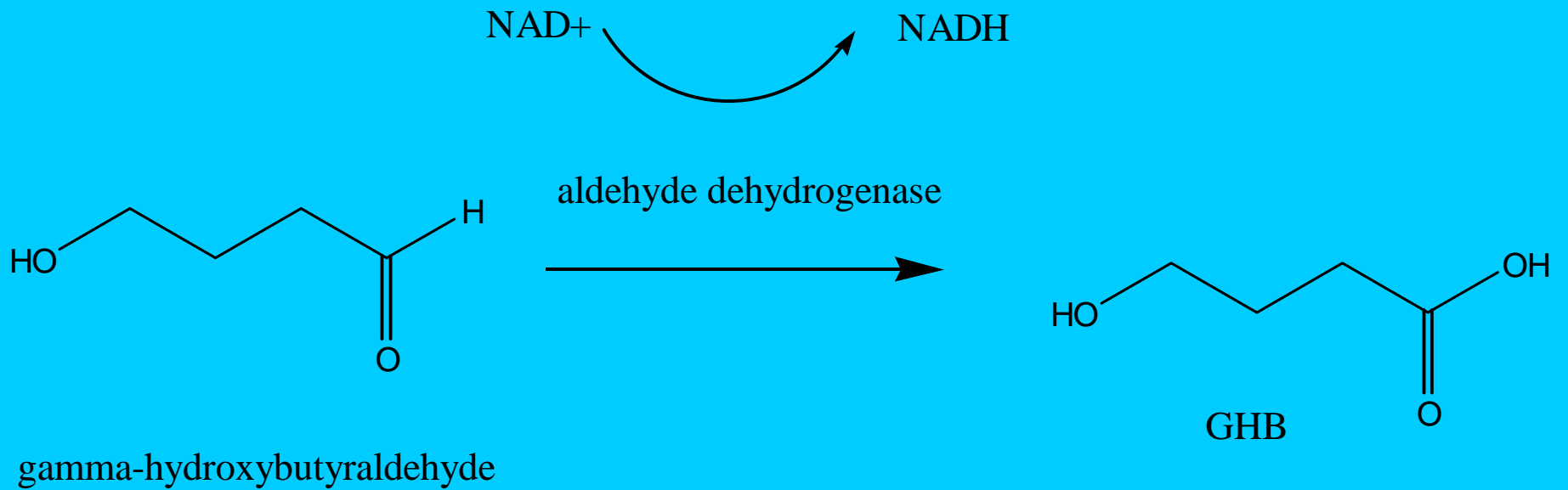
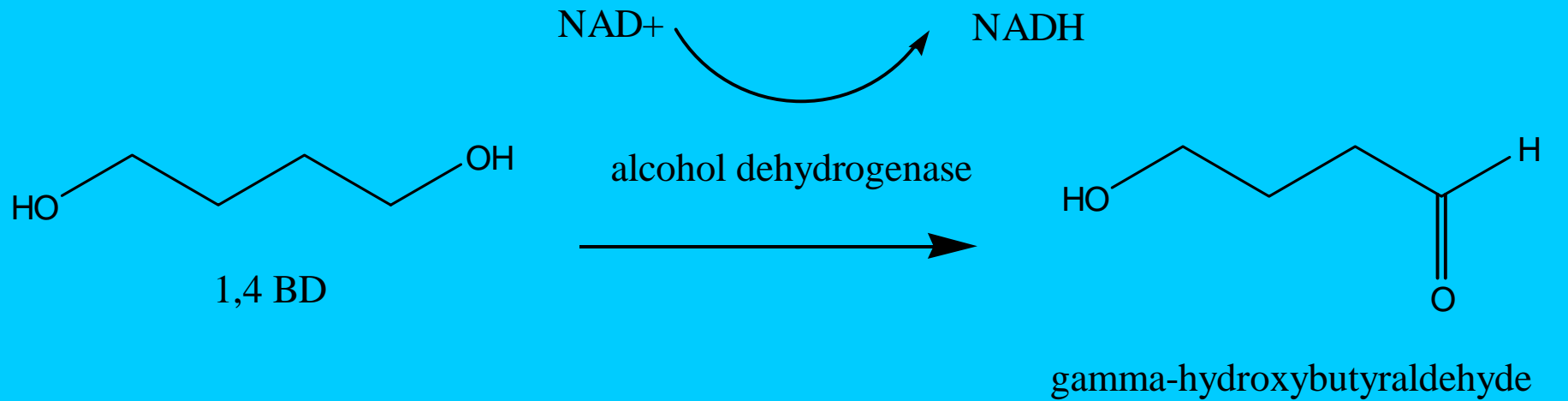
Effects of 1,4 BD

- Same as GHB
- 1,4 BD is converted to GHB in vivo by two enzymes - 1st alcohol dehydrogenase (ADH) then aldehyde dehydrogenase
- Many chemical synonyms like GBL

Chemical Synonyms for 1,4BD

CHEMICAL SYNONYMS FOR 1,4 BD	
butane-1,4-diol	sucol B
1,4-butylene glycol	1,4 tetramethylene glycol
1,4-dihydroxybutane	butylenes glycol
diol 14B	tetramethylene 1,4-diol

Metabolism of 1,4 BD



1,4 BD metabolism

- The activity of ADH towards 1,4BD is similar to its activity towards ethanol
- Ethanol is a competitive inhibitor of 1,4BD to GHB metabolism
- Aldehyde dehydrogenase in second step is inhibited by disulfiram like in ethanol metabolism
- Liver, brain, kidney and heart metabolize 1,4BD to GHB with liver showing the greatest conversion capacity per gram of tissue (Irwin, R.D. 1996)

Typical user GHB, GBL or 1,4BD

- Body builder type (20-30yrs - WM)
- Teenagers - euphoric effects, aphrodisiac, low calorie high
- Rave party attendees - euphoric and as a “come down” from MDMA use
- Individuals intent on committing sexual assault or incapacitation of others in order to facilitate criminal activity

High Times Mag. 1998

- Hey, Lisa I'm bringing the Gamma-G so I hope your ready to get wild tonight
- I've been partying for two nights thanks to Gamma-G and I'm still ready for more sex, fun and more Gamma-G
- I hope you didn't eat because we're doing some Gamma-G when we meet the girls at the party
- Bro me and Gamma-G are already at the party and feeling great but if you have some wild chicks fired up, I'm there.

200gm
10 Dose Bottle
\$89.95
Trial Size
\$15.00

GAMMA-G

"The Ultimate Experience"

Hey, Lisa. I'm bringing the Gamma-G, so I hope you're ready to get wild tonight.

I've been partying for two nights thanks to Gamma-G and I'm still ready for more sex, fun and more Gamma-G.

I hope you didn't eat, because we're doing some Gamma-G when we meet the girls at the party.

Bro, me and Gamma-G are already at the party and feeling great, but if you have some wild chicks fired up, I'm there.

Extreme Science has finally delivered an ultimately unique way of having fun.

- ecstatic pleasure
- exotic and seductive situations
- extreme euphoric pleasure
- hilariously funny times
- mind blowing rushes

Gamma-G is a must for yourself, a party, or just hanging out with your friends.

To order, and other product info:
 1-800-841-2600 or you can visit on the world wide web at
www.gamma-g.com

To Order by Mail: Send address cash/check/money order (Check held 2-3 weeks) to: 3506 Hwy 6 #033, Sugar Land, TX 77478. Tel: 800-841-2600.

Name: _____

Address: _____

City/State: _____ Zip: _____

Phone (optional): _____

All major credit cards accepted.

Qty.	Item#	Price	Total
<small>Add \$4.95 S&H or \$5 for priority mail.</small>			
<small>Texas residents, add 8.25% sales tax.</small>			GRAND TOTAL

*Wholesale and distributor pricing available Genesis Caps and Joker Caps also available

Advertisement for GBL Product, High Times November 1998
Image by Murple, © 2001 Erowid.org

“Typical” user GHB, GBL or 1,4BD

- GHB ‘N Juice
- GHB ‘N XTC

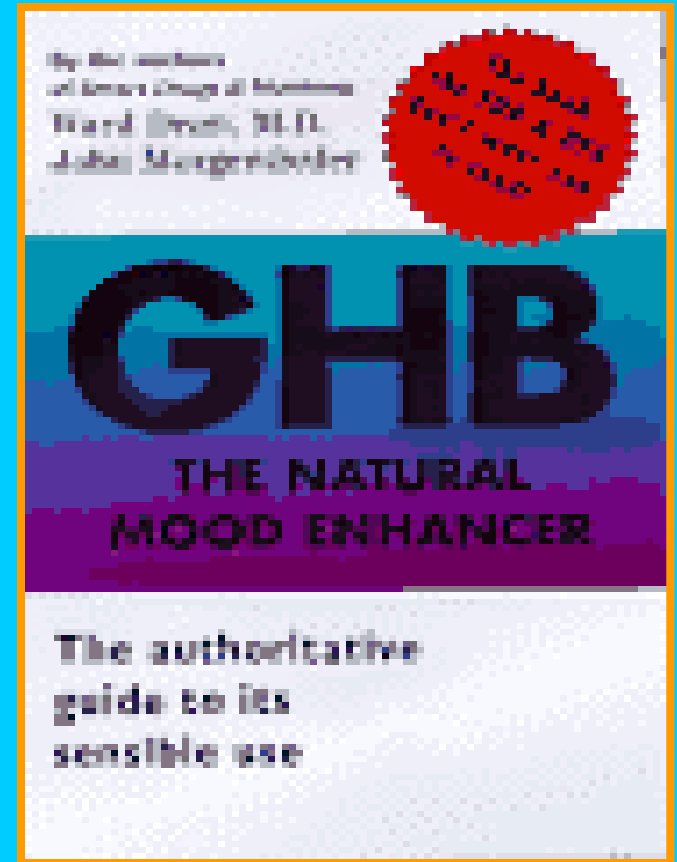
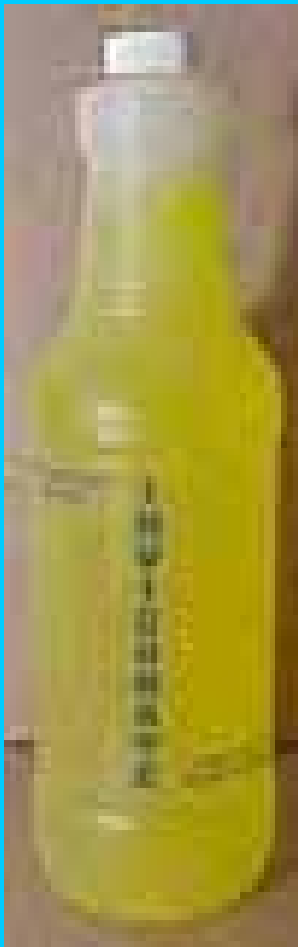


Where can you get GHB, GBL or 1,4BD

- Illicit manufacture - solutions can have a salty, soapy or plastic taste, A base (NaOH, KOH) plus GBL = GHB
- Illicit powder has a chemical odor (moth ball like)
- Commercial Preparations of GBL or 1,4BD
- Internet and health food supplement distributors, hardware stores, www.chemicalkits.com
- Use chemical synonyms to disguise ingredients on label, also add fruit type scents - i.e. blueberry scented cleaner



GHB on the Internet



- http://www.erowid.org/entheogens/ghb/ghb_faq2.shtml
- <http://www.lycaeum.org/drugs/synthetics/ghb/ghbsynth.html>
- <http://www.ghbinfo.com/GETIT.HTM>



GHB Kit

Anonymous Photographer, © 2000 Erowid.org

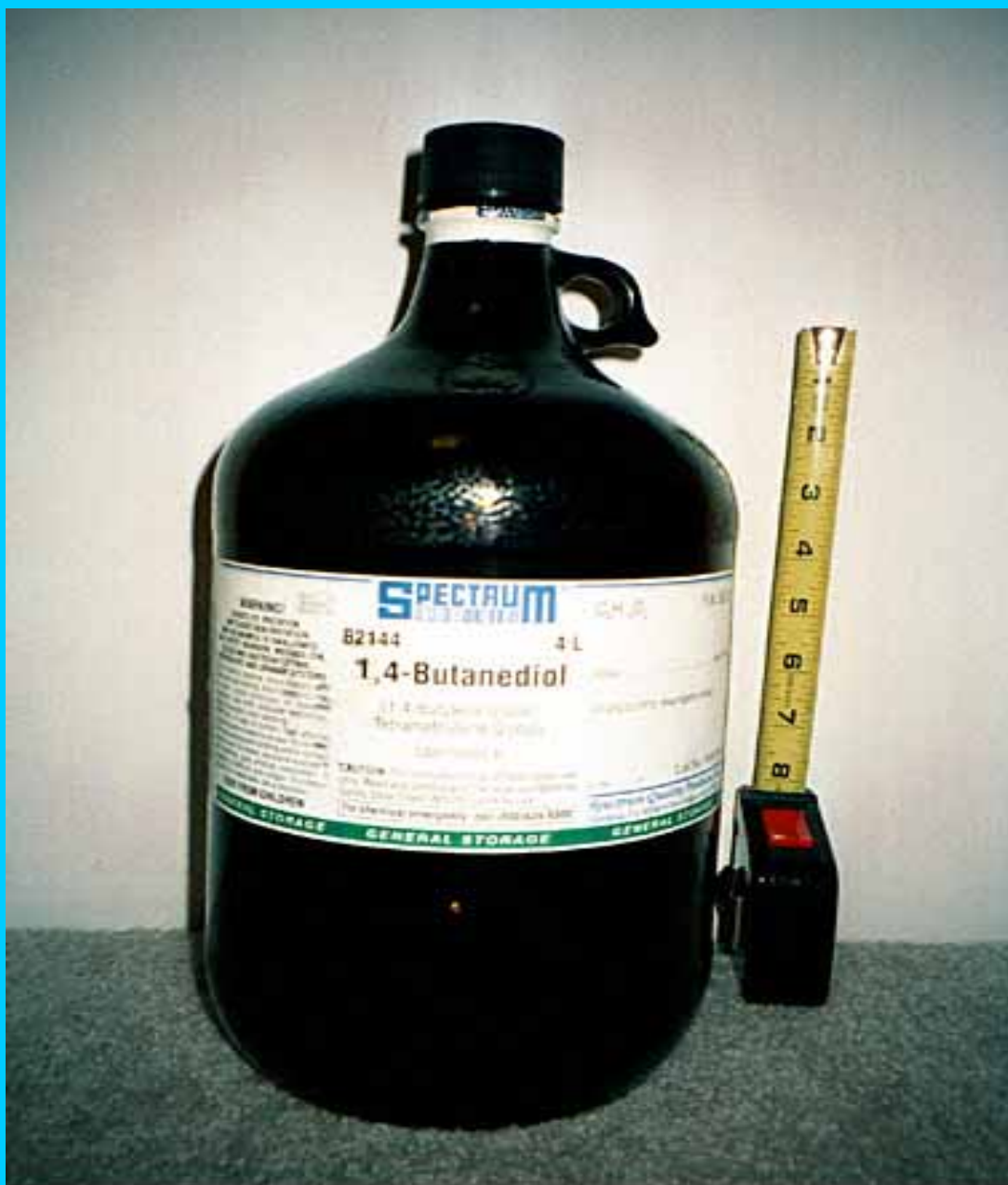


- GBL
Product



when sleep is safe and desired. Ensure that those around you are aware that you may be unarousable and that this is normal. Higher doses will result in proportionally longer periods of deep sleep. Excessive doses may result in sweating, muscle spasms, vomiting, bedwetting, and diarrhea. Unless drugs or alcohol have been taken with Blue Nitro the only treatment necessary is to SLEEP IT OFF! A call for help may result in uninformed emergency medical personnel using expensive, unnecessary and potentially dangerous methods of arousal.

- KEEP OUT OF REACH OF CHILDREN
- DO NOT COMBINE WITH OTHER DRUGS OR ALCOHOL



Jug of 1,4-Butanediol

Photo by Murple, © 2000 Erowid.org



Not GHB ?!

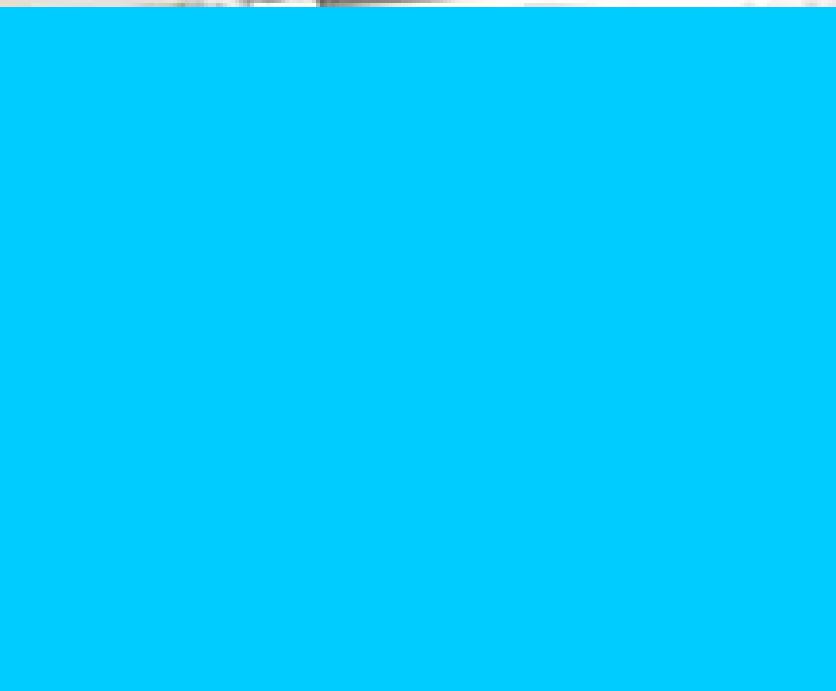
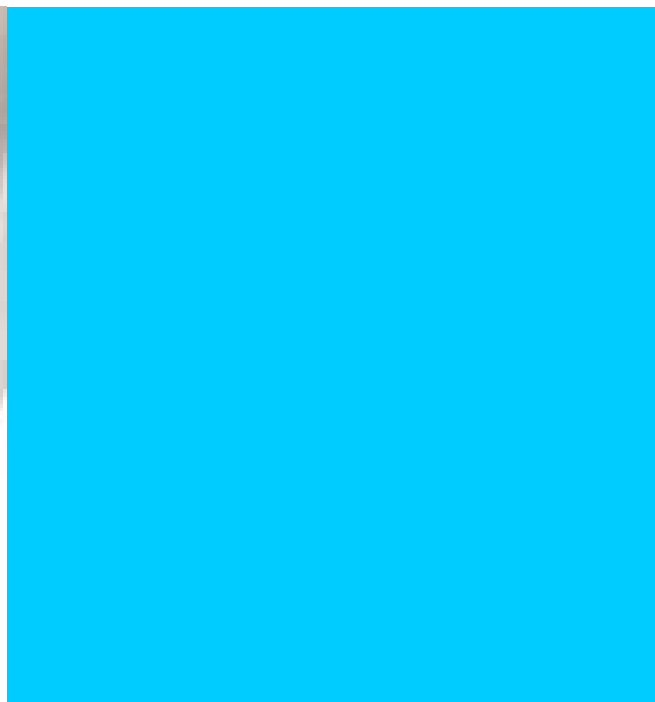
- SDI-Labs
- “Prosexual Secretagogue”
- <http://webcast.com/s-labs/ghb.html>
- Proposed to enhance secretion of norepinephrine and testosterone
- yohimbine HCl and 4-androstenediol
- daily use by men but only 2x’s a week for women



Dose Response of GHB

- < 1 gram - mild relaxation, decreased anxiety, enhanced social ability
- 1 - 2 grams - strong relaxation
- 2 - 4 grams - induce sleep
- 4 - 8 grams - very deep sleep
- > 10 grams - very deep, long lasting sleep leading to coma

(this assumes pharmaceutical grade GHB and a non tolerant individual)



Therapeutic GHB Dose

- Alcohol withdrawal syndrome -
25 - 50mg/kg every 12 hours (~2 - 4g for 170lb man)
- Prolonged deep sleep - 75 -100mg/kg (~6 - 8g for 170lb man)
- Anaesthetic induction - > 100mg/kg (> 8g for 170lb man)
- Narcolepsy Rx - up to 9g divided into 2 doses
4.5g doses given 4 hour apart

GHB blood levels in anesthesia

- 16 adults - correlation of state of consciousness with GHB level
- Doses: 50, 75, 100 and 165 mg/kg
- Range of 100 mg/kg dose = 234 to 520 mcg/ml
- 12 of the 16 patients required intubation
- Not necessarily those who received the highest GHB doses (Helrich et al.1964)

GHB levels vs arousal states in humans in mcg/mL

Sleep State =	Awake	Light Sleep	Medium Sleep	Deep Sleep
GHB $\mu\text{g/mL}$ *	0 - 99	63 - 265	151 - 293	244 - 395

Table 1 * values converted from micromoles/L

Contrast blood levels seen in Alcohol Withdrawal Syndrome Treatment

- Doses of 25 and 50 mg/kg
- 25 mg/kg range = 24 to 88 mcg/ml - ~ 2g for 170lb man
- 50 mg/kg range = 51 to 158 mcg/ml - ~ 4g for 170lb man

Patients do not display sedative effects

- Chronic treatment - tolerance develops
(Ferrara et al.1993)

Compare CSF levels GHB in humans

- Infants - 0.26 to 0.27 mcg/ml
- Children - 0.11 to 0.13 mcg/ml
- Adults - 0.02 to 0.03 mcg/ml

- Children with myoclonic type seizure disorder highest conc. GHB in CSF 0.78 to 0.97 mcg/ml
(Snead et al. 1981)

Outcome of toxic dose

- Respiratory support (ventilation)
- Stabilize and maintain patient vitals
- Wait it out
- Usually a full recovery without long term effect (acute use)
- IF care is given (ventilation) prior to prolonged hypoxia

Why is GHB so Dangerous??

- Very steep dose response curve
- Metabolism pathway exhibits zero order kinetics (like ethanol) - biphasic elimination
- Illicit manufacture introduces impurities and variations in potency
- Consumed with other drugs mainly ethanol
- Addictive - produces tolerance and withdrawal

GHB is addictive

- Chronic use of GHB over long periods of time
- GHB produces tolerance and withdrawal
- Symptoms include; insomnia, muscular cramping, tremor, anxiety and in some cases a temporary schizophrenic-like state with hallucinations
- Reports of addicts consuming in excess of 100 grams a day (Dyer et al. 2001)

Research Evidence

Suggests Cellular Tolerance

- **Humans**
 - Chronic treatment results in GHB dependence; discontinuation leads to GHB withdrawal (Addolorato et al., 2000, Dyer et al., 2001)
 - Cellular cross-tolerance from chronic ethanol use – alcoholics receiving GHB show less impairment than normal controls with similar doses and blood concentrations (Ferrara et al., 1993; Helrich et al., 1964)

Interactions

- GHB potentiates CNS depressant effects of other CNS depressants ($1 + 1 = 3$)
- Lab data with rats supports the theory that GHB potentiates ethanol (Marinetti & Commissaris)
- Not by saturation of a common metabolic pathway (GHB has its own dehydrogenase)
- Cross tolerance between ethanol and GHB



When sleep is safe and desired. Ensure that those around you are aware that you may be unarousable and that this is normal. Higher doses will result in proportionally longer periods of deep sleep. Excessive doses may result in sweating, muscle spasms, vomiting, bedwetting, and diarrhea. Unless drugs or alcohol have been taken with Blue Nitro the only treatment necessary is to SLEEP IT OFF! A call for help may result in uninformed emergency medical personnel using expensive, unnecessary and potentially dangerous methods of arousal.

- KEEP OUT OF REACH OF CHILDREN
- DO NOT COMBINE WITH DRUGS OR ALCOHOL

+



©NITRO

Combinations

- 40% - GHB alone
- Combinations:
- **86% - with ethanol**
- 20% - with stimulants
- 18% - with hallucinogens
- 3% - with sedatives

(incidence of GHB use with MDMA is increasing)

Methodologies for GHB Analyses

Detection:

- GC/FID
- GC/MS(EI)
- GC/MS(CI)
- *No screening test - yet*

Extraction:

- Liquid:Liquid
 - Solid Phase
 - Solid Phase
- Micro-extraction
(SPME)

Analysis of GHB

- Commonly involves conversion to Gamma Butyrolactone (GBL) by acid incubation
- GHB can be directly analyzed for but must be derivatized
- Separation and detection by GC/FID or GC/MS

Analytical Concerns - I

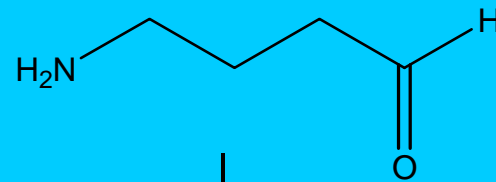
- GHB is rapidly absorbed and eliminated (dose dependent)
- Timely specimens a must (12 hr urine - 6-8 hr blood - Hoes et al. 1980, Kavanaugh et al. 2002)
- Endogenous ante-mortem GHB can be as high as 6 or 7 mcg/mL in urine (Gibson et al. 1990, LeBeau et al. 2002, Elian 2002)
- GHB has been documented to increase post mortem (normal decomposition process) always analyze urine too
- GHB or GBL is O.K. *No in vivo* conversion of GHB to GBL* or 1,4BD *(Possible exception stomach contents)

Putrescine



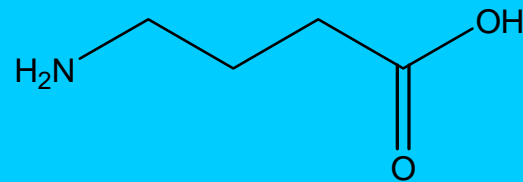
Diamine oxidase

4-aminobutyraldehyde

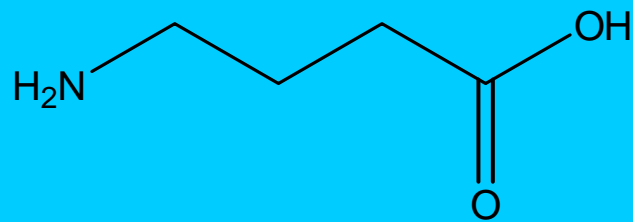


Aldehyde dehydrogenase

GABA

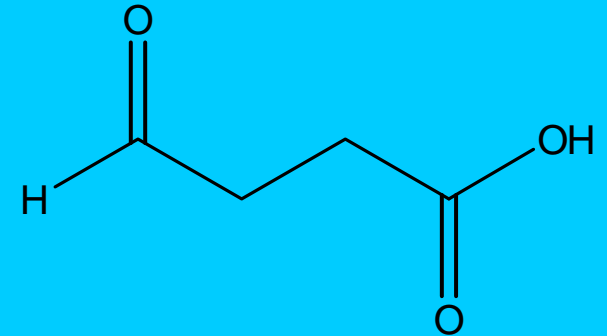


Formation of GHB from GABA

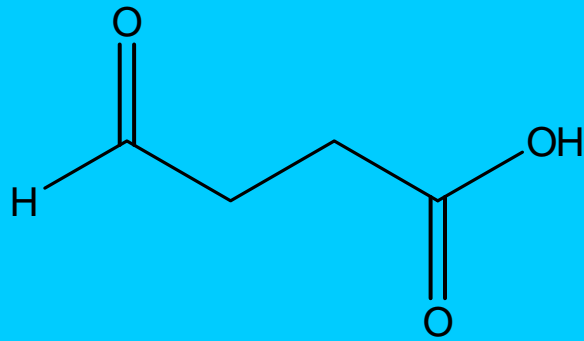


GABA

GABA transaminase

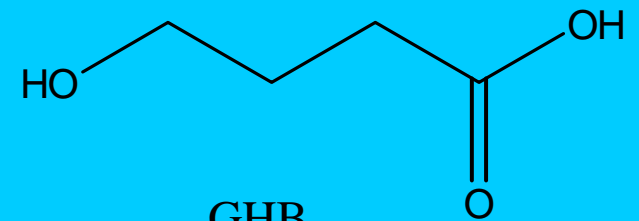


succinic semialdehyde



succinic semialdehyde

SSA reductase



GHB

Analytical Concerns II

- Urine analysis - urea can interfere (Di-TMS deriv.)
- GHB is a metabolite of other compounds and as a result can be detected even if no GHB is consumed
- Tetrahydrofuran - not normally for human consumption but an overdose has been reported with the GHB metabolite detected and quantified (Cartigny et al. 2001)
- 5-Fluorouracil - consumed as the anti-cancer drug, Ftorafur, the GHB metabolite has been documented (El Sayed and Sadee 1983)

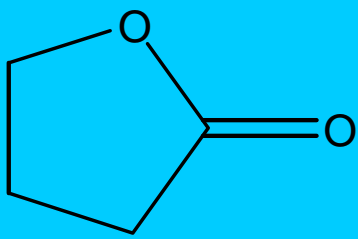
Does more advanced decomposition mean greater GHB levels postmortem?

- Not necessarily
- Although GHB is formed in the putrescine metabolic pathway - it is not the end product
- Enzymes involved are ubiquitous in nature
- Several strains of bacteria can utilize GHB as a food source or produce GHB from succinate (Sohling and Gottschalk 1993 and 1996, Henne et al. 1999)

GHB and GBL are dynamic

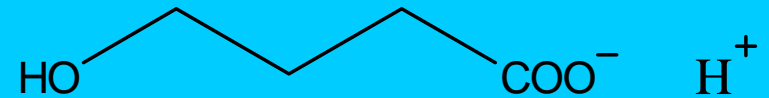
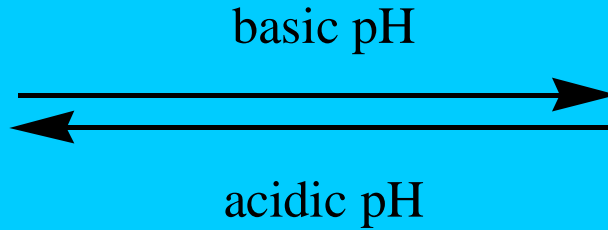
- GHB and GBL co-exist
- GHB predominates when the pH is > 4.72 OR when the lactonase enzyme is present
- GBL predominates when the pH is < 4.72 and no lactonase enzyme is present
- Can GBL exist in stomach contents or urine if only GHB was consumed??
- YES - Why????
- How fast conversion occurs depends on time, temp. and pH of matrix (Ciolino et al. 2001, Marinetti et al. AAFS 2002)
- GBL can be detected in blood if extremely high GBL dose is ingested (enough to saturate lactonase enzyme)

GHB and GBL Equilibrium



GBL

pH < 2



GHB

pKa = 4.72

1,4 BD and GHB

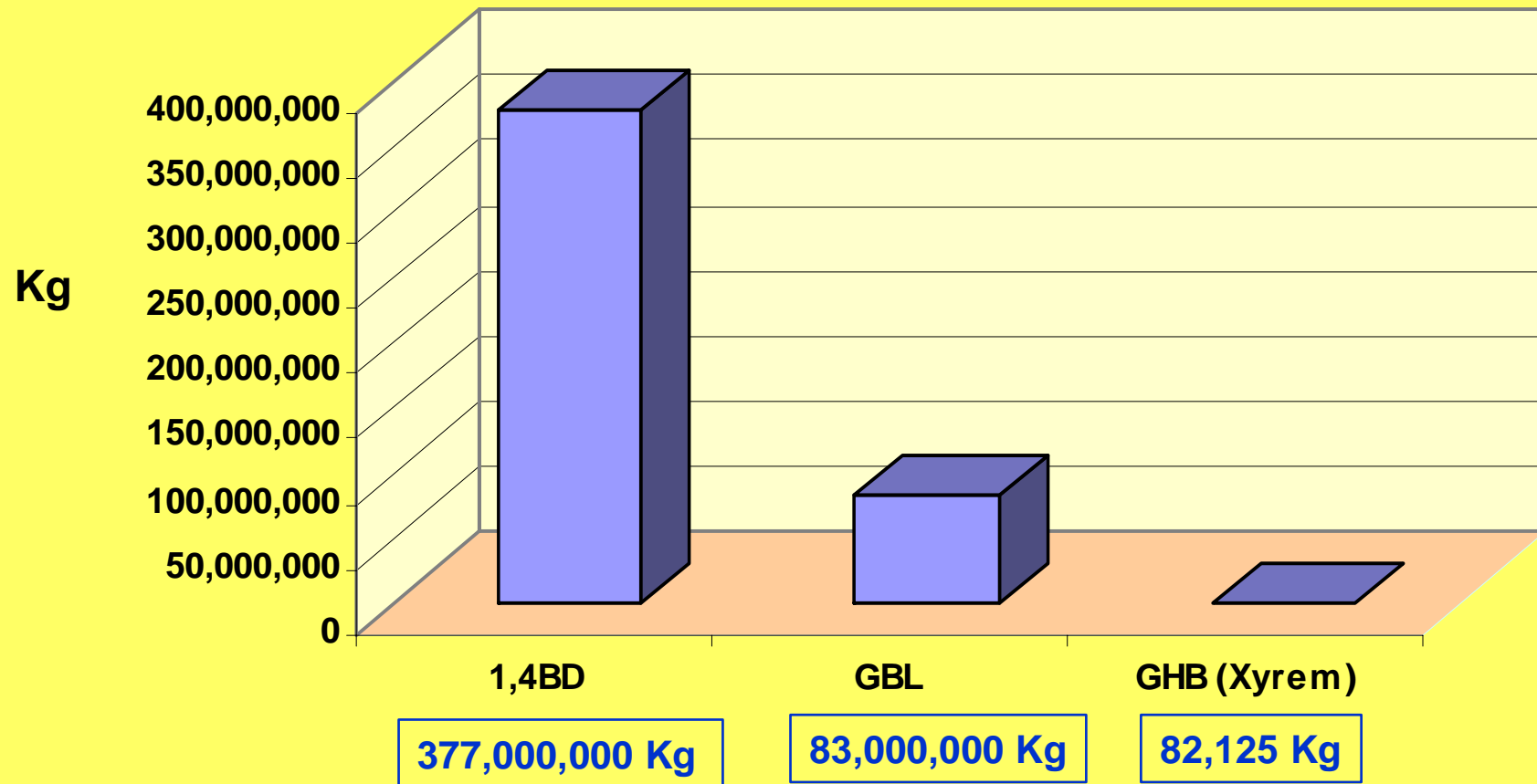
- Unchanged 1,4BD may be detected in a biological specimen
- Depends upon how much is ingested and the time interval since the ingestion (McCutcheon et al. AAFS 2000) and (Kraner et al. SOFT 2000)
- Metabolic enzymes can become saturated
- 1,4 BD is not in chemical equilibrium with either GHB or GBL

GHB scheduling has curbed illicit GHB use??

- No - metabolic precursors (GBL and 1,4BD) are far more prevalent than GHB - even when it is sold for narcolepsy treatment

GHB, GBL and 1,4-BD

Comparison of Production Quantities



Case Studies (DUI)

- 12/98 JR “Renewtrient”
 - analyzed by FBI and LA County ME
 - curb to curb driving, low speed chase for miles, vomiting inside truck, incoherent, no sense of location, name or destination, could only repeat questions
 - blood 135 $\mu\text{g/ml}$ GHB
- 9/99 JR “Renewtrient”
 - DRE observations: sweating, delayed pupil response, unable to track pen, vertical and horizontal nystagmus
 - urine 1900 $\mu\text{g/ml}$ GHB

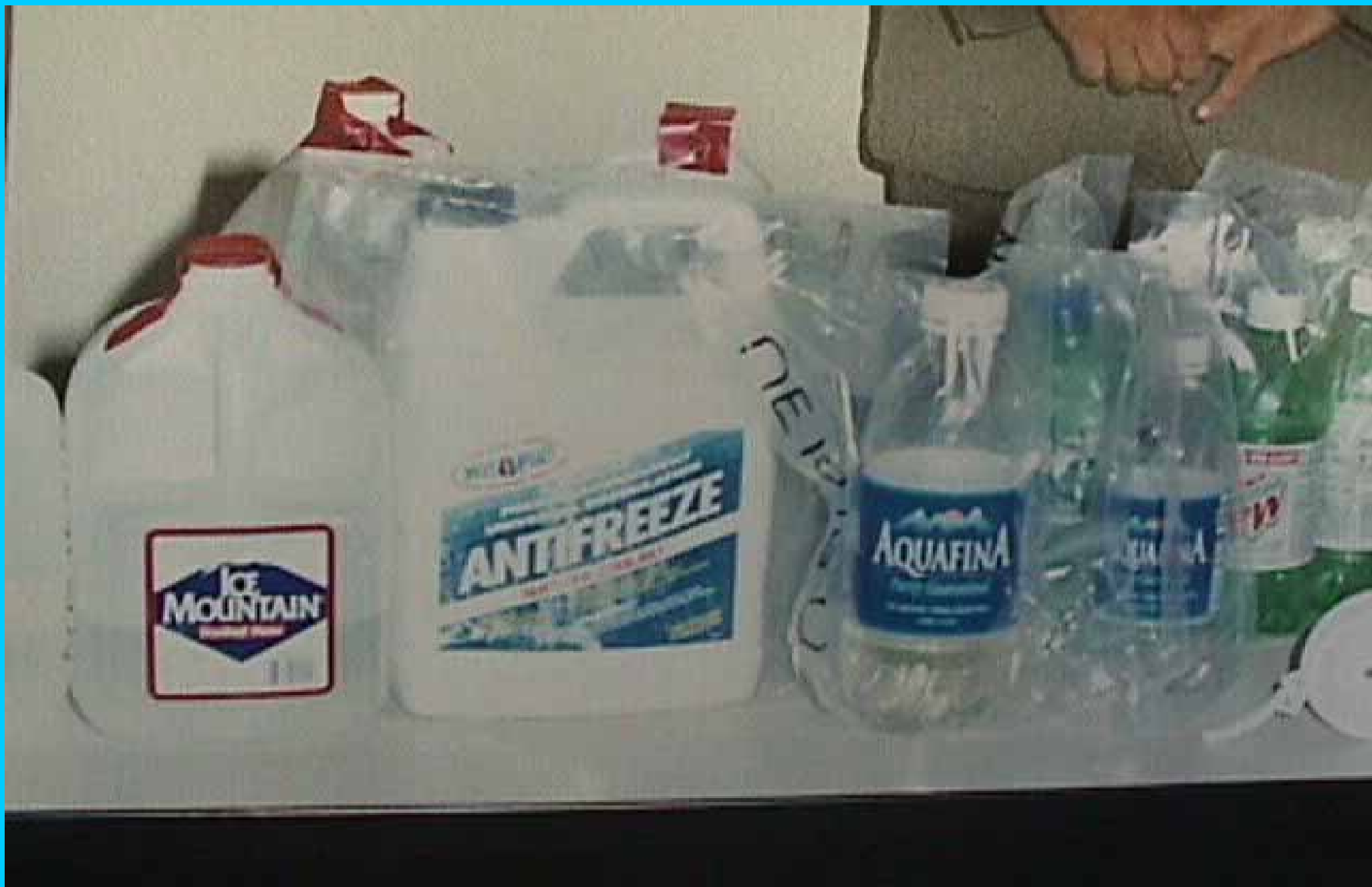
Case Studies Cont (DUI)

- 5/99 HM
 - found outside vehicle, passed out along roadside
 - hospitalized
 - blood 361 $\mu\text{g/ml}$ GHB
- 12/99 HM
 - blood 85 $\mu\text{g/ml}$ GHB
 - phenobarbital

Case Studies Cont (DUI)

- 8/99 AB
 - 8 gallons GHB in trunk
 - manufacture of GHB
 - blood 86 $\mu\text{g/ml}$ GHB
 - urine 830 $\mu\text{g/ml}$ GHB
 - severe withdrawal upon incarceration
- 9/99 AB passenger in vehicle
 - driver's blood 87 $\mu\text{g/ml}$ GHB
 - during arrest, AB accidentally grabbed water bottle and drank, profuse vomiting, hospitalization
 - 2 gallons GHB in trunk









Case Studies Cont (Non-Fatal Overdose)

- Subject unconscious, rushed to ER
 - subject ingested FUBAR (BD)
 - blood collected approximately 9 hrs after hospitalization
 - blood 13 $\mu\text{g/ml}$ GHB
 - urine 272 $\mu\text{g/ml}$ GHB
 - possible longer duration of detection with BD?

Case Studies

- Non fatal GHB - WCMEEO Case
 - 29yrs white male- weight lifter
 - Hit by car while walking down middle of expressway at night
 - History of chronic GHB use reported by his mother - she wanted him committed - acting strangely
 - Fight with girlfriend - she dropped him off on side of expressway and left

Case Studies

- Non fatal GHB - WCMEO Case

- Postmortem Blood (heart) 111 mcg/mL
- Postmortem Blood (femoral) 100 mcg/mL
- Vitreous Humor 310 mcg/mL
- Bile 76 mcg/mL
- Postmortem Urine 400 mcg/mL

- MOD - accident or suicide????

Case Studies Cont (Overdoses)

- Fatal overdose - WCMEO case 1
 - 22yrs white male
 - found dead 5 hours after mother had last seen him
 - No evidence of vomiting
 - Suspected to be a regular GHB user
 - Autopsy - enlarged heart (400g), thickened left ventricular wall, lung weights of 600 and 650g
 - Toxicology - negative except for GHB

Case Studies Cont (Overdoses)

- Fatal overdose - WCMEO case 1
 - Heart Blood - 66 mcg/mL
 - Femoral Blood - 77 mcg/mL
 - Vitreous Humor - 85 mcg/mL
 - Urine - 1260 mcg/mL

Case Studies Cont - Overdoses

- Fatal overdose - WCMEO case 2
 - 15yrs white female
 - Was slipped GHB/GBL in a drink (mountain dew)
 - Friends transported her to ER when she became violently ill (vomiting) then unresponsive
 - Clinically dead on arrival - pronounced 14 hours later
 - Autopsy - mild edema of brain (1400g), lungs were 900 and 800g with edema and evidence of aspiration pneumonia
 - Toxicology negative except for GHB and lidocaine

Case Studies Cont (Overdoses)

- Fatal overdose - WCMEO case 2

Ante-mortem Blood - 510 mcg/mL

Ante-mortem Urine - 2300 mcg/mL

14 hours later....

– Postmortem Blood - 15 mcg/mL

– Postmortem Urine - 150 mcg/mL

– Postmortem Vitreous - 127 mcg/mL

Case Studies Cont - Overdoses

- Fatal overdose - WCMEO case 3
 - 30yrs white female
 - Found dead in bed - rigor was present
 - Purge from mouth - feces on body and bed
 - Autopsy - negative
 - Toxicology negative except for GHB and fluoxetine (prozac)

Case Studies Cont (Overdoses)

- Fatal overdose - WCMEO case 3
 - Postmortem Blood - 310 mcg/mL
 - Postmortem Urine - 2100 mcg/mL
 - Postmortem Blood - 0.013 mcg/mL fluoxetine

Case Studies

- Fatal overdose - WCMEO case 4
 - 40yrs male
 - found dead in bed
 - became hyperactive and aggressive
 - used “steroids” to increase muscle mass
 - tranquilizers to calm down
 - ecstasy user
 - recently depressed over financial problems
 - autopsy - ASCVD

Case Studies

- Fatal overdose - WCMEO case 4

	mcg/mL
– Postmortem Blood GHB -	510
– Postmortem Urine GHB -	3200
– Vitreous GHB -	332
– Bile GHB -	393
– Postmortem Blood - trace of diazepam (valium), paroxetine (paxil) and MDA (MDMA metabolite) (all < 0.12 mcg/mL)	

Case Studies Cont - Overdoses

- Fatal overdose
 - bar drinking with the guys
 - “friend” had some “good stuff” in Gatorade bottle
 - passing around capfuls, decedent took large swig of bottle
 - back at the bar, passed out, loss of bladder/bowel control
 - blood 400 $\mu\text{g/ml}$ GHB, BAC 0.22 g/dl

 - vitreous fluid (1 week after embalming)
212 $\mu\text{g/ml}$ GHB, VAC 0.12 g/dl

Case Studies Cont

- Fatal overdose
 - barbecue doing “G” with friends
 - man at the barbecue decides to try and incapacitate other males so he can “have his way with the women”
 - he substitutes diluted GBL for full strength GBL and encourages others to drink it
 - several get ill including decedent
 - by the time they realize what’s happening decedent is already died - he has vomited and lost bladder and bowel control
 - blood 390 mcg/ml GHB

GBL and 1,4BD are the only
GHB-like products to be
concerned about???

- **“Tranquili-G (patent pending) is a breakthrough sleep-aid/mood-enhancer.”**

**“Excellent Valium
Alternative
Great for Deeper Sleep
Each 4oz bottle contains
24-48 servings.”**



“The Science:

Tranquil-G is the trade name for 4-pentanolide (patent pending). Upon ingestion, Tranquili-G is converted via lactonase catalyzed hydrolysis -- the same mechanism by which GBL is converted to GHB (1) -- to the potent GHB analogue, 4-methyl-GHB (see *). 4-methyl-GHB has been found to bind to the GHB receptor with 15% GREATER affinity (FALSE see Bourguignon et al. 1988 and 2000) than GHB itself (2). Gamma-hydroxybutyrate exerts its effects through specific GHB receptors (3), thus consumption of a precursor to a compound which binds to the GHB receptor would be expected to result in physiological and psychological effects quite similar to those produced by GHB, such as sedation, reduction of anxiety, and stimulation of growth hormone release.” (NOT KNOWN)

(author's comments)

source: www.musclesoft.com

Chemical Synonyms for GVL

- 4-pentanolide
- dihydro-5-methyl-2-(3H)-furanone
- 4-hydroxypentanoic acid lactone
- 4-hydroxyvaleric acid lactone
- gamma-methyl-gamma-butyrolactone
- 4-methyl-gamma-butyrolactone
- gamma-pentalactone
- tetrahydro-5-methyl-2-furanone
- dihydro-5-methyl-2-furanone
- 5-methyltetrahydro-2-furanone
- 4-hydroxyvaleric acid gamma lactone
- 5-methylbutyrolactone
- 4-valerolactone
- 4-hydroxypentanoic acid gamma lactone
- 4-methylbutyrolactone

What is Gamma-valerolactone??

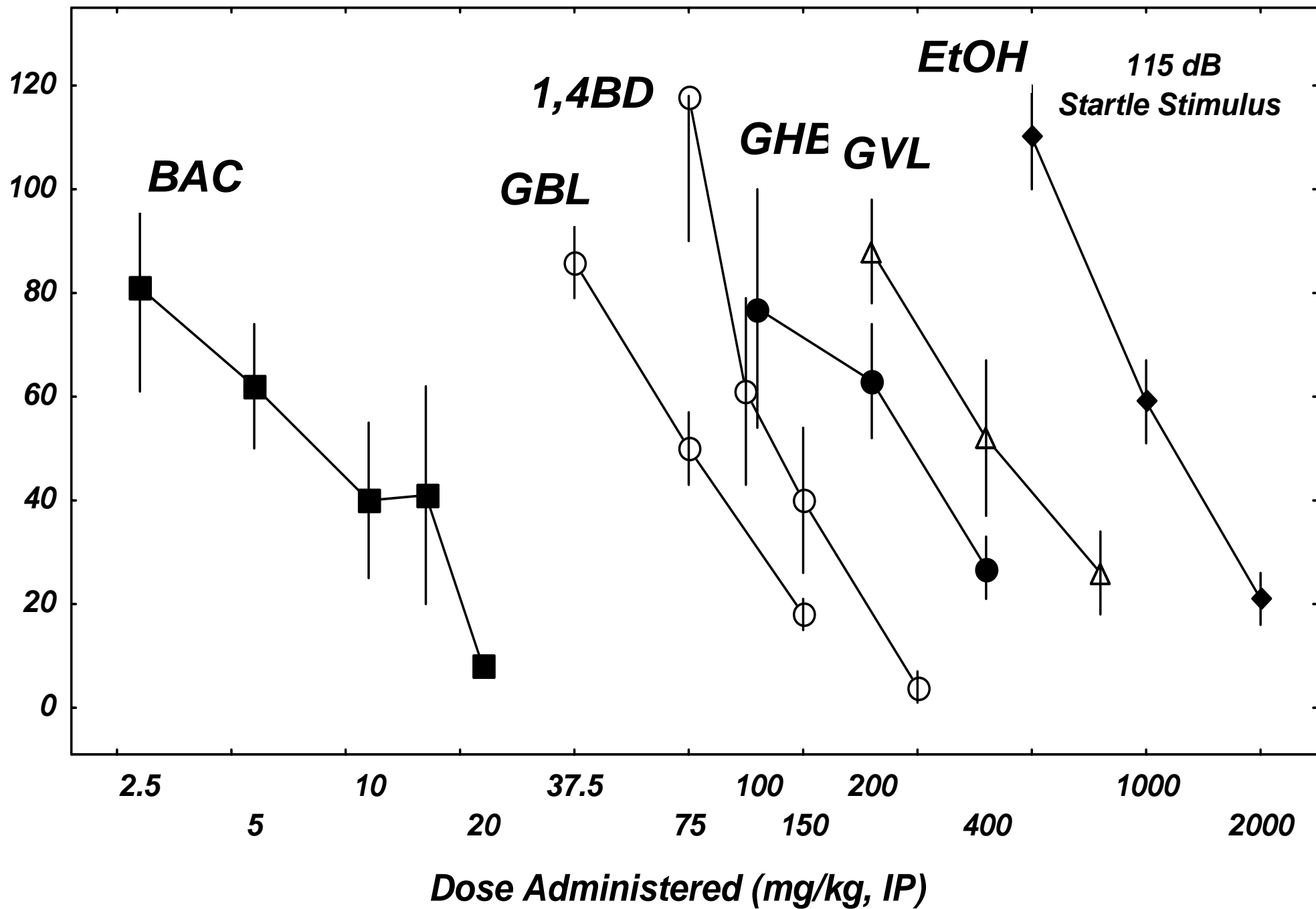
- FDA approved food additive (flavoring agent or adjuvant)
- Safe for human consumption at 0.0009322 mg/Kg per day per person
- Approximately 1100 pounds used per year
- Potentially found in 199708 beverages
- LD50 8.8g/Kg oral in rat, 2.5g/Kg oral in rabbit - (J Ind. Hyg. Toxicol. 1945)

Source: U.S. Food and Drug Administration Food Additive Safety Profile - Docnum=1551

Pharmacology of GVL

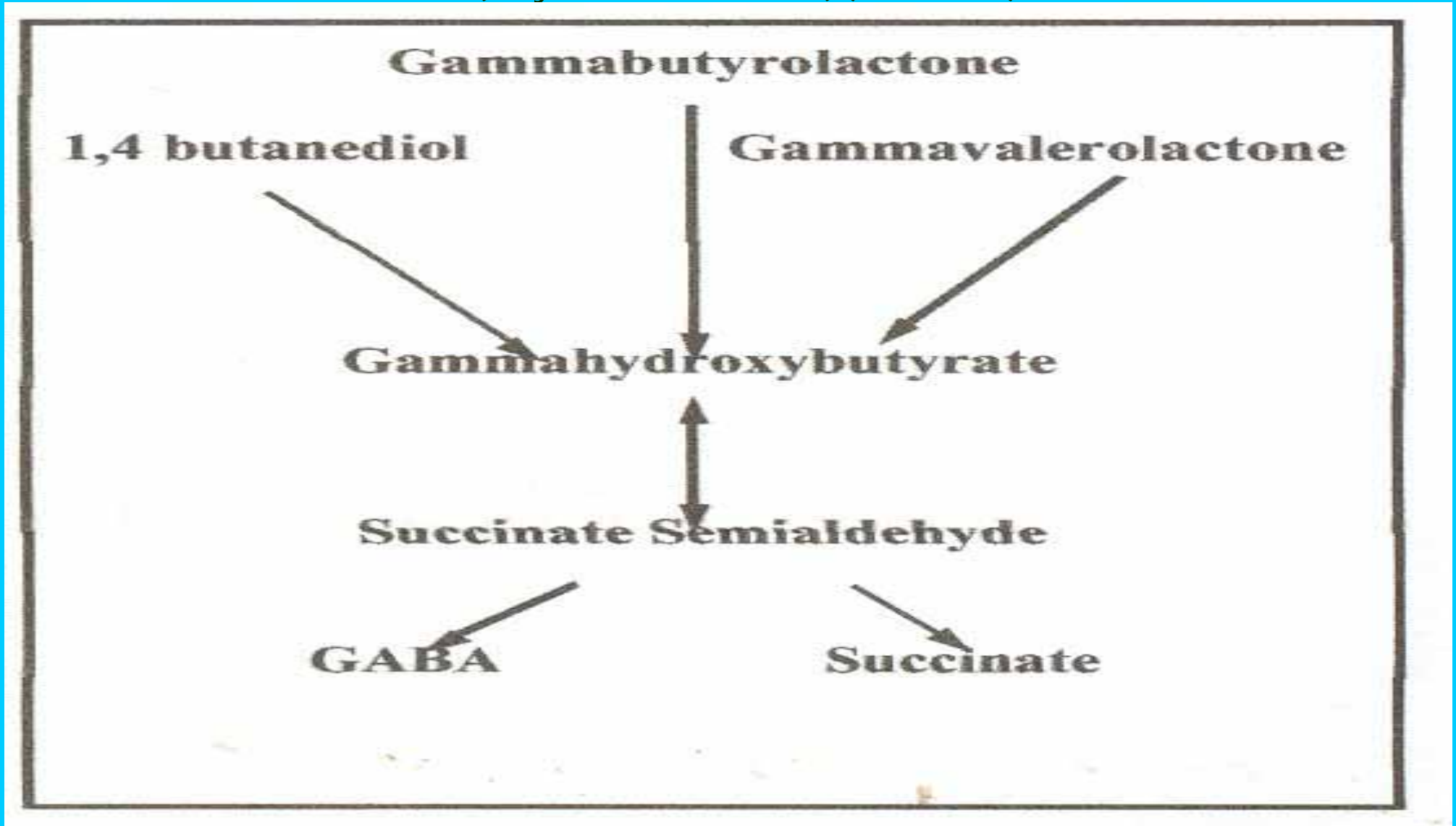
- Comparison of GVL to GHB, GBL and 1,4 BD in the rat shows it to have similar sedative effects but lower potency (Marinetti and Commissaris - SOFT ERA 2002)

Startle Amplitude (% Control)

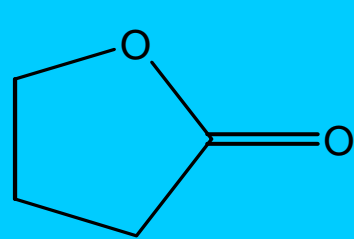


Is GVL converted to GHB?

(Doyon, ToxAlert 17(3):1, 2000)

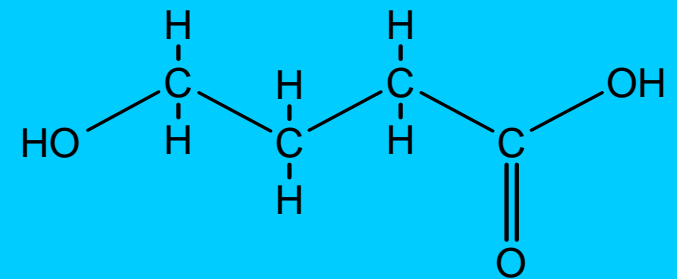


In vivo: blood and liver
(Fishbein and Bessman 1966)

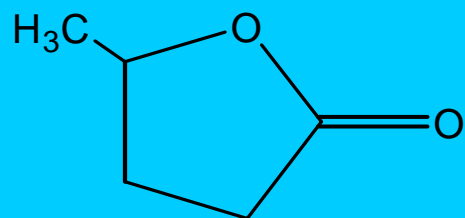


Gamma Butyrolactone

lactonase

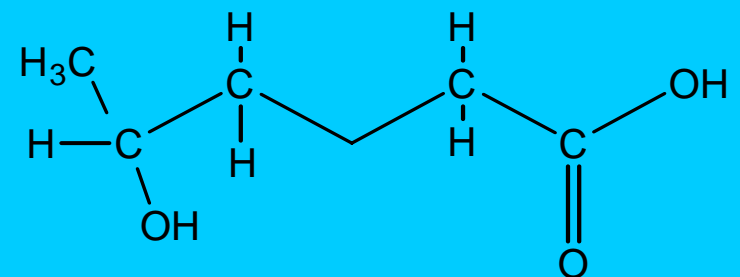


GammaHydroxybutyric Acid



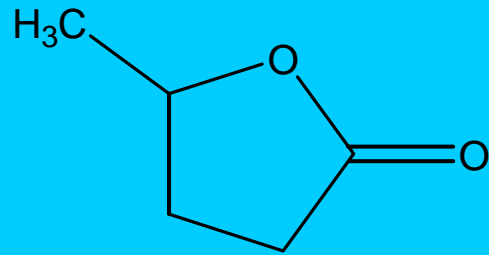
Gamma Valerolactone

lactonase



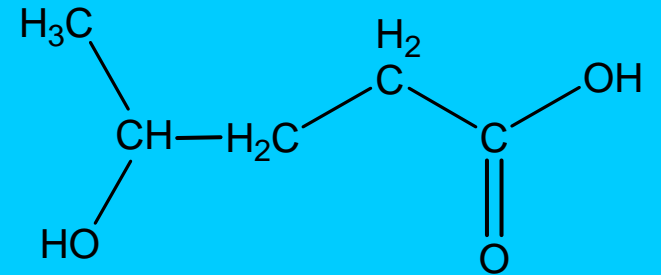
Gamma-Methyl-GHB

In Vivo:



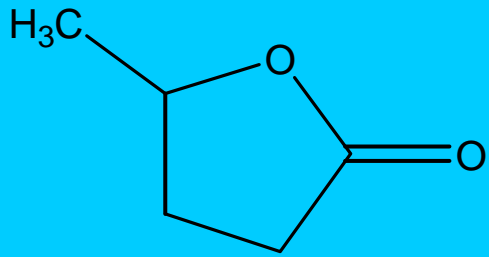
Gamma valerolactone

lactonase



Gamma methyl, gamma hydroxyvalerate

In Vitro:

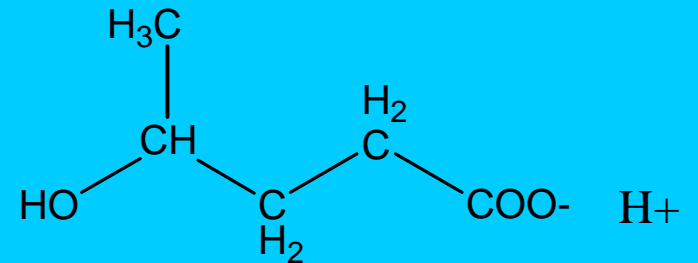


GVL

basic pH



acidic pH



Gamma hydroxy valeric acid

pKa = 5.72

Conclusions I

- Interpretation of GHB concentrations are complex
- Recommend at least 10mcg/mL as cut-off between endogenous and exogenous ante-mortem urine GHB
- Clears from the body quickly (10-12 hours urine, 6-8 hours for blood)
- More commonly seen in human performance toxicology as opposed to postmortem
- Tolerance can occur with chronic use
- Usually consumed with other drugs (EtOH, MDMA etc...)
- GHB and GBL are dynamic - be mindful of matrix pH and type (I.e. urine vs blood vs aqueous)

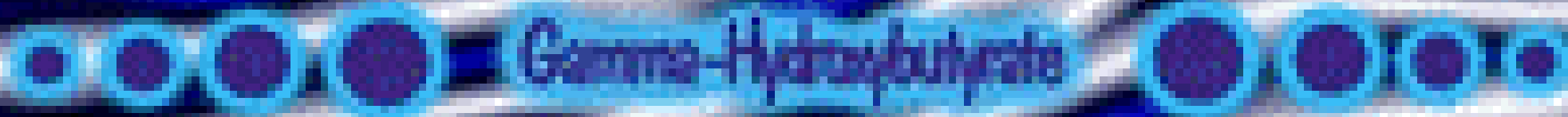
Conclusions II

- No “fatal” concentration range
- Postmortem GHB production can be a problem
- Evaluate multiple matrices and get a good history
- Also production can occur in some hospital type blood tubes
- citrate (other tube types require evaluation)

- Due to popularity as industrial solvents, GBL and 1,4 BD are here to stay
- Watch for new “replacements” for GHB - GVL

GHB

Gamma-Hydroxybutyrate



- Gamma-hydroxybutyrate; Molecular, Functional and Clinical Aspects, Editors; G.Tunnickliff & C. Cash, Taylor and Francis, London and New York, 2002.
- Marinetti, L.J. Benzodiazepines and GHB: Detection and Pharmacology, Editor S. Salamone, Humana Press, Totowa, N.J. 2001, pp. 95-126, 2001.
- Couper F.J., and Marinetti L.J., GHB Monograph, Forensic Science Review, Vol. 14(1/2), Central Police University Press, Taipei, Taiwan and Birmingham, Alabama, 2002, pp. 101-121.