LABORATORIO NACIONAL DE PREVENCIÓN Y CONTROL DEL DOPAJE-CONADE
WADA ACCREDITED LAB

Elimination study of Clenbuterol by consumption of meat contaminated and a pharmaceutical preparation

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Why is Pharmacokinetics Important?

Knowledge of pharmacokinetic data about a drug tells physicians:

- What dose to give
- How often to give it
- How to change the dose/route in certain medical conditions
- How some drug interactions occur

Why does only a fraction of the total dose reach its target?

How should we dose (route) and how many times (frequency) to maintain drug at target (efficiency)?





Why is Pharmacokinetics Important?

Knowledge of pharmacokinetic data about a drug tells anti-doping scientists:

- ✓ If and how an athlete was using a prohibited substance including possible/probable sources
- ✓ Understanding the window of detection
- ✓ When an athlete may have started and stopped using
- ✓ Whether single and multiple doses were ingested
- ✓ Whether an athlete was receiving a performance benefit
- ✓ Other possible routes of administration/ingestion
- ✓ Help to establish intentional or unintentional use which can determine the athlete's degree of fault





Basic Terminology

Pharmacokinetics……what the body does to the drug

Pharmacodynamics……what the drug does to the body

If a drug is going to have an (performance-enhancing) effect in the body it needs to be present:

In the right place

At the right concentration

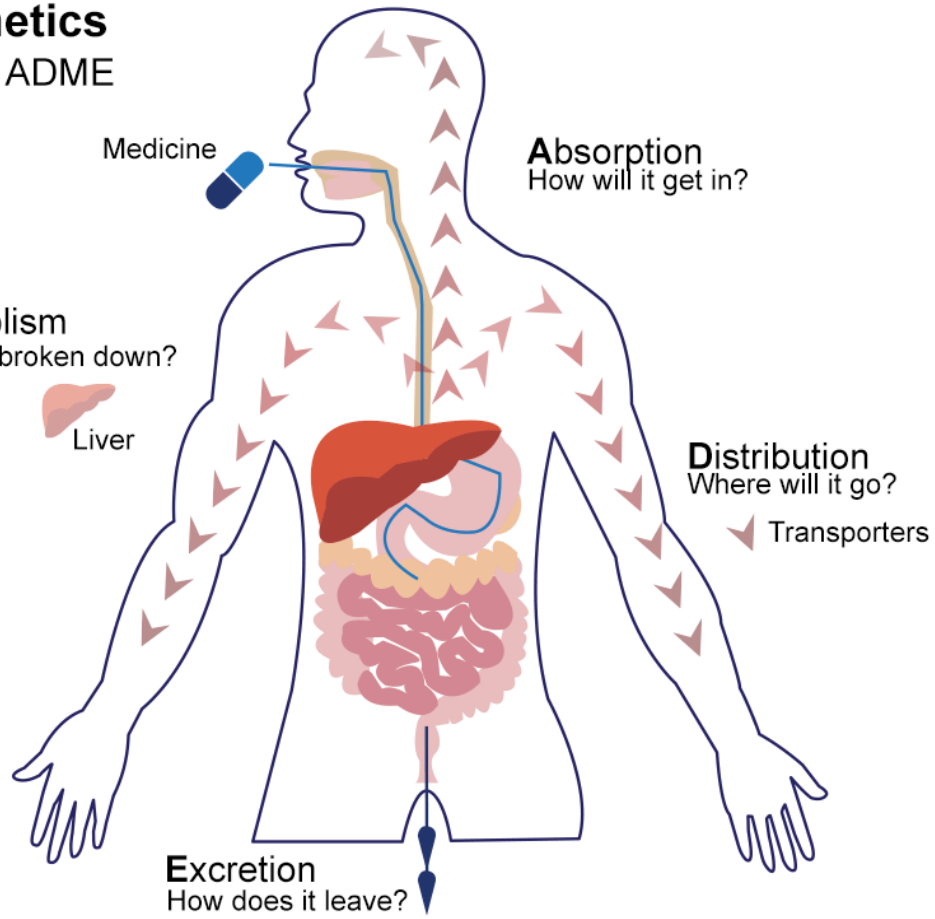
For the right amount of time





Pharmacokinetics

The principles of ADME

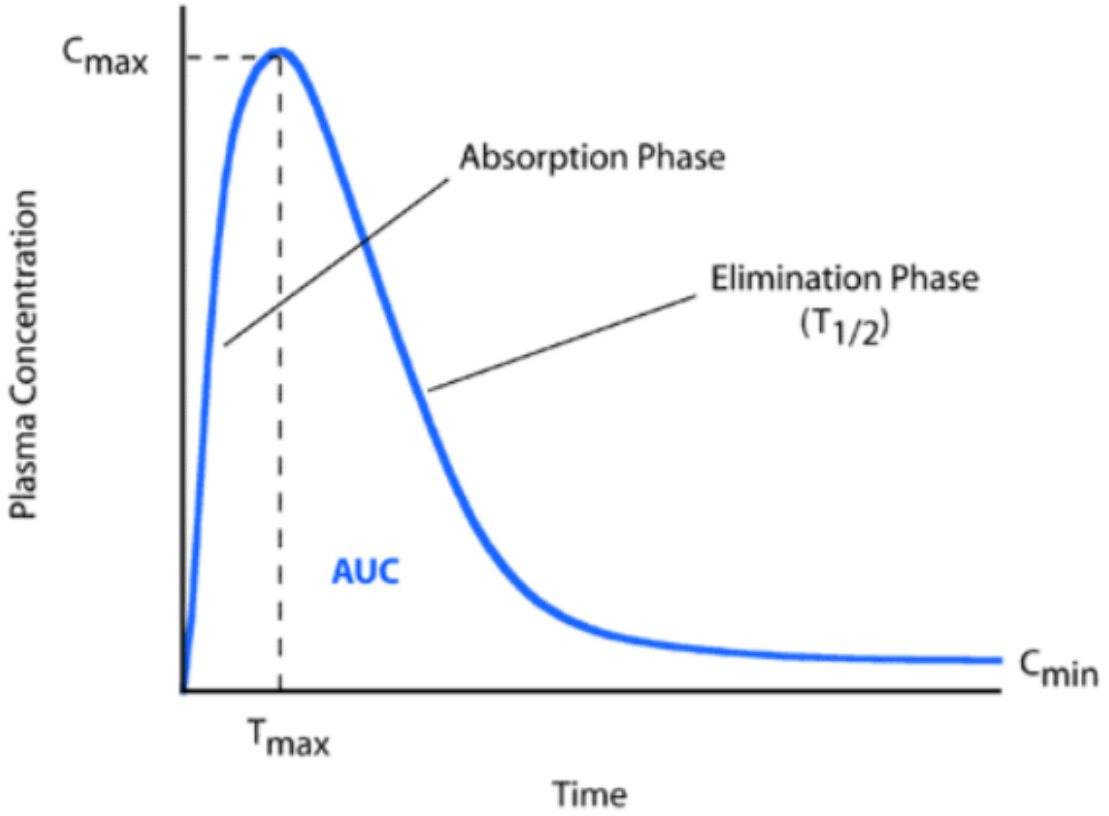


EUPATI
European Patients' Academy on Therapeutic Innovation
www.eupati.eu





What happens to a drug when taken orally (or IV, IP, SC)?

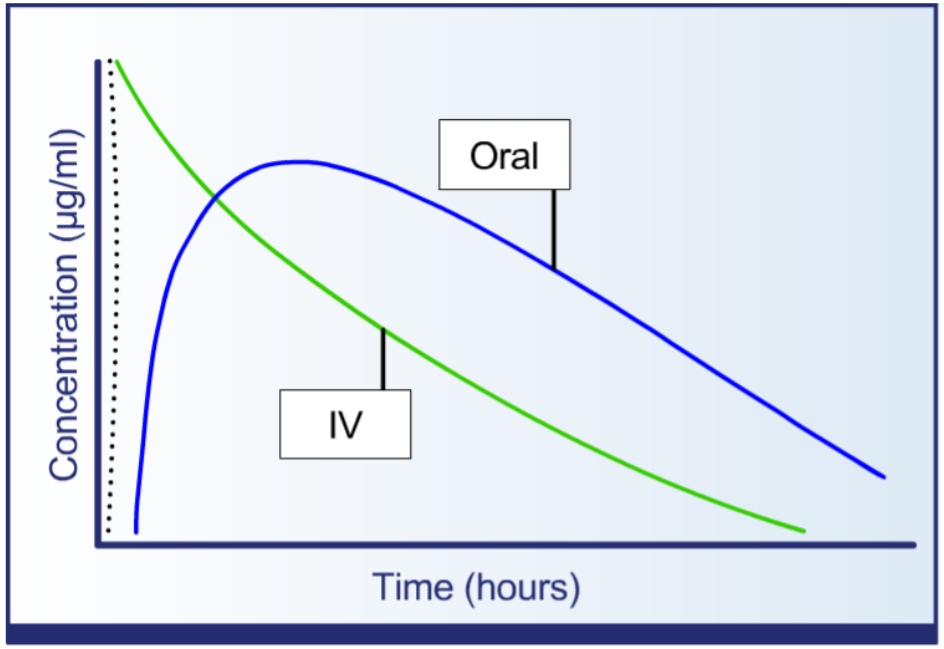


- A favorable PK profile is vital to the therapeutic success of a drug
- Drug must be able to reach its intended target

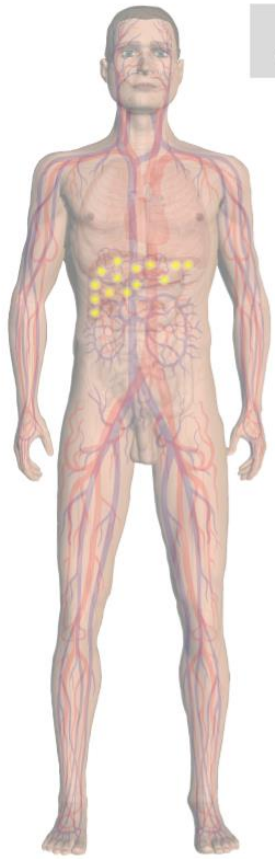


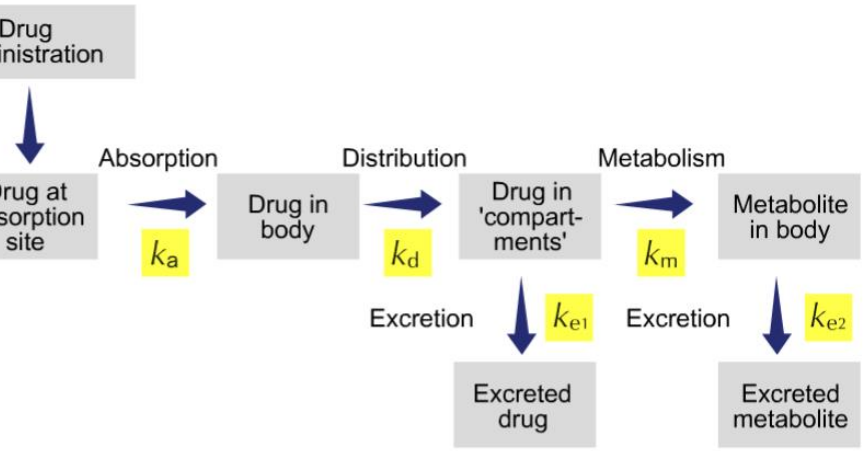


Drug Absorption and Elimination



Rate of change of drug in body = Rate of absortion - Rate of elimination





Rate constant: *k*

A proportionality constant in the rate equation that describes the relationship between the rate of a process and the concentration of a drug.

Rate equation (first-order):
rate of a process = k x drug concentration

Rate constants are important because they determine the time course of drug absorption, distribution, metabolism and elimination.





- Maximum observed concentration (C_{max})
- Time to reach C_{max} (t_{max})
- Area under the curve (AUC)
- Clearance (CL)
- Bioavailability (F)
- Volume of Distribution (V)
- Volume of Distribution at steady-state (V_{ss})
- Half-life ($t_{1/2}$)
- Mean Residence Time (MRT)





Goals for this study

Administration of contaminated meat with Clenbuterol as well as a pharmaceutical preparation to males and females with the following goals.

- ✓ Measure the concentration of clenbuterol in urine samples after exposition (single and repeat) of contaminated meat.
- ✓ Can determine the time when no clenbuterol is detected.
- ✓ Isomeric profile of clenbuterol.





What is Clenbuterol?

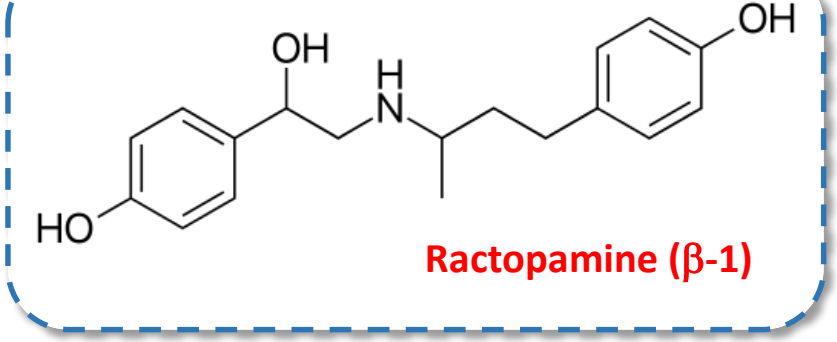
Hydrochloride is a powerful bronchodilator that is used to treat breathing disorders like asthma.

Clenbuterol has also been noted for having a strong anabolic effect; however, things are not quite like they a… potential anabolic effect, this has caused many to use the compound in hopes of gaining lean tissue. Commo…, many steroid users have used it as an anabolic protective agent during their post cycle therapy (PCT). There is, however, a problem with this type of use; it doesn't work. Studies have shown that Clenbuterol has the ability to promote anabolic activity in animals. There have been several studies that have shown the anabolic activity of rats to increase when Clen is administered. However, there is no data that supports such anabolic activity provided when used by human beings. In fact, it has generally been proven useless in this regard as it pertains to human beings. When it comes to human Clenbuterol use, use as a bronchodilator and thermogenic are the only suitable purposes.

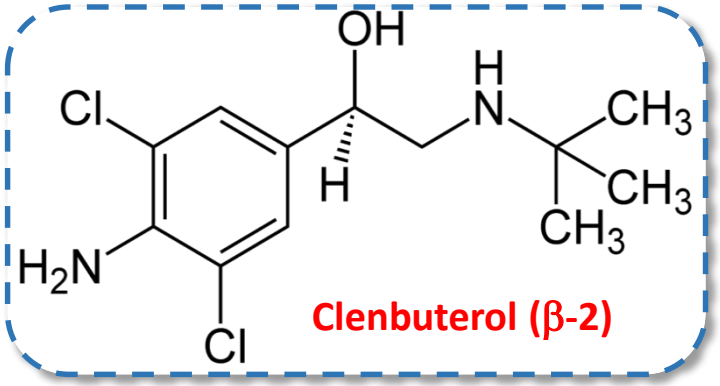




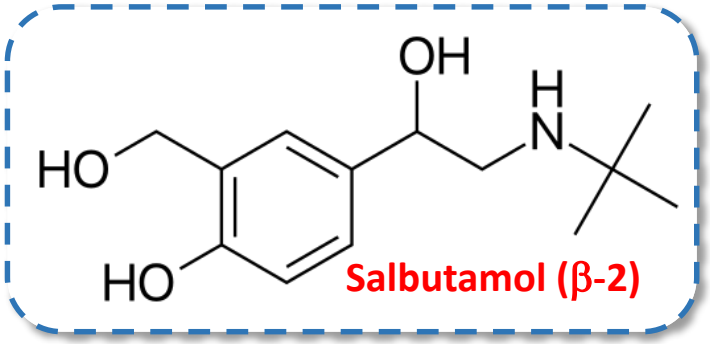
β-adrenergic agonist



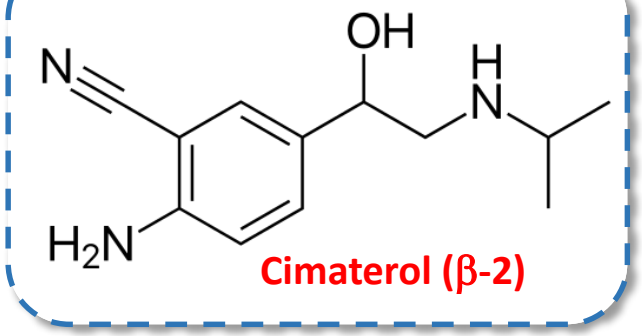
Ractopamine (β-1)



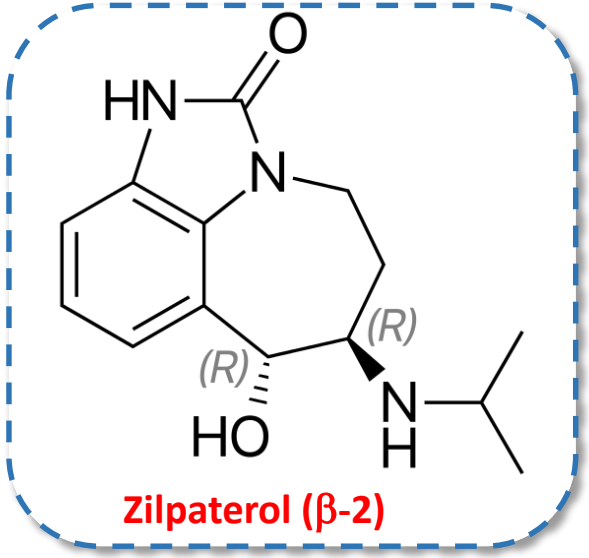
Clenbuterol (β-2)



Salbutamol (β-2)



Cimaterol (β-2)



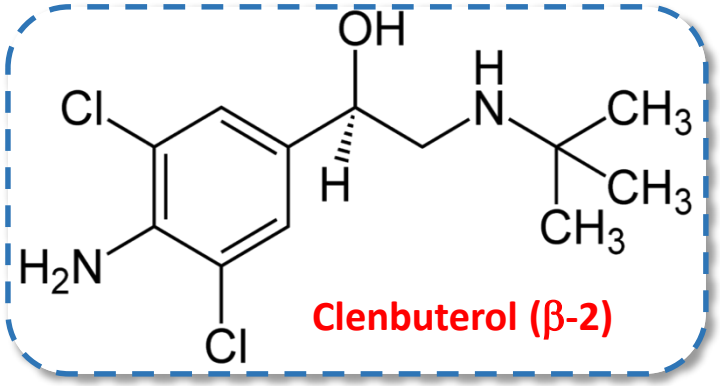
Zilpaterol (β-2)





Relevance to Anti-Doping

- ✓ An importan number of AAF by Clenbuterol
- ✓ A banned substance in sports
- ✓ Athletes involved in AAF



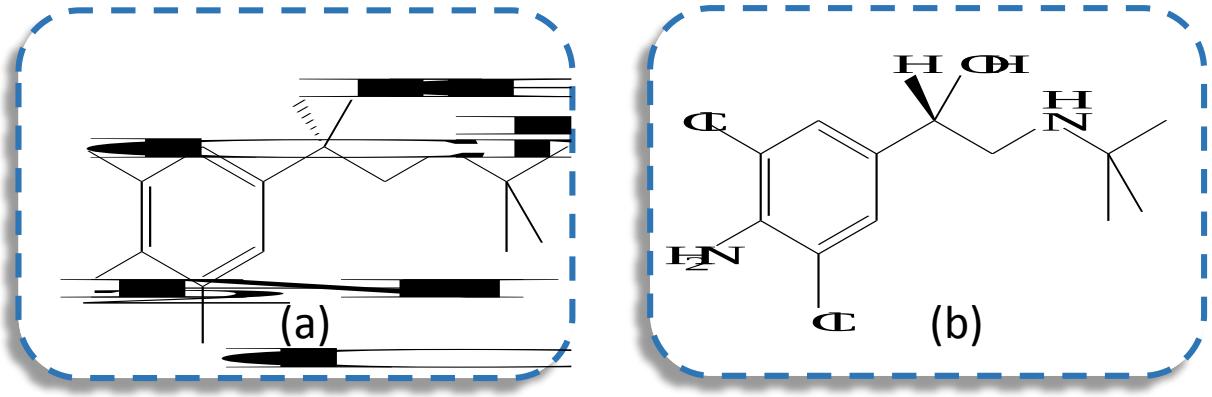
Clenbuterol (β-2)



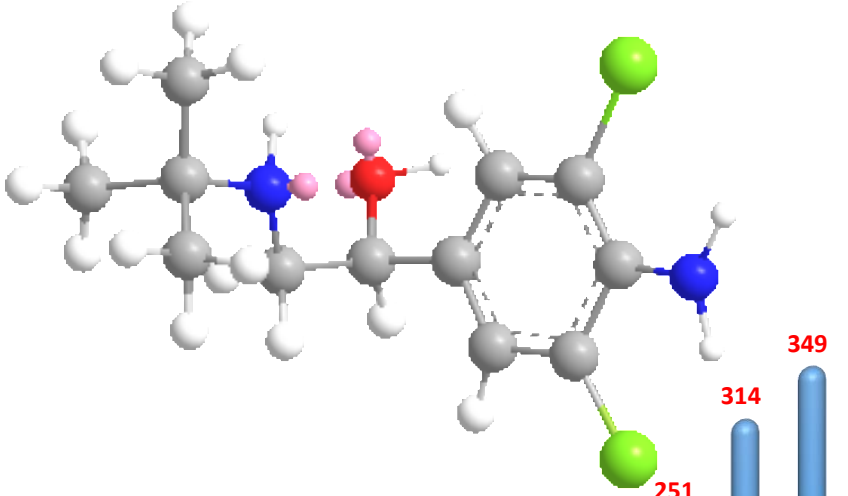


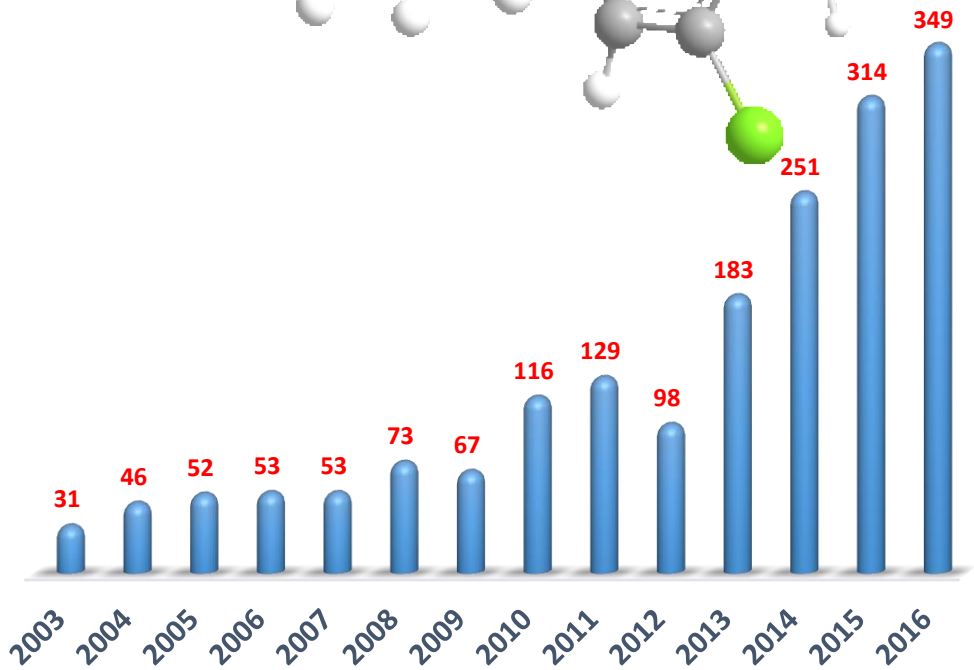
Clenbuterol is a substance used for increasing of performance in athletes,

Hydrochloride is a powerful bronchodilator that is used to treat breathing disorders like asthma.



Chemical structures of clenbuterol enantiomers:
(a) *R*-(-)-clenbuterol and (b) *S*-(+)-clenbuterol.



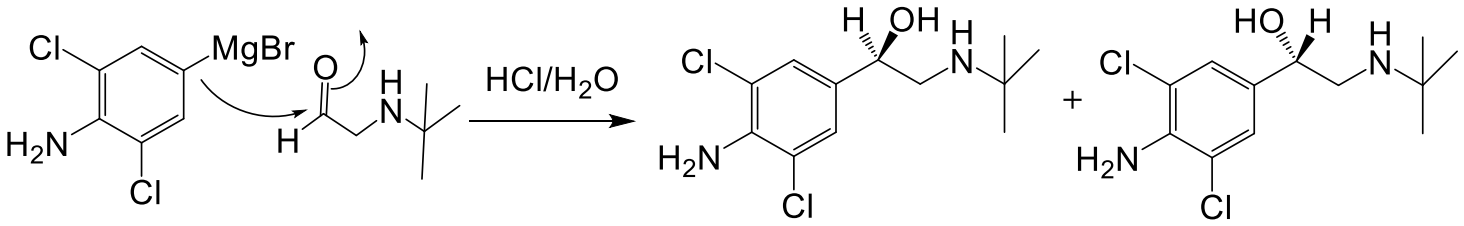


https://www.wada-ama.org/en/resources/laboratories/anti-doping-testing-figures





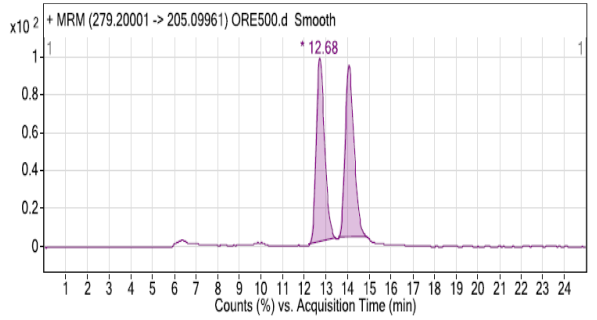
Synthesis of racemic clenbuterol



***R*-(-)-clenbuterol** **+** ***S*-(+)-clenbuterol**





Resolution of R-() and S-(+)- enantiomers of clenbuterol in pharmaceutical preparations and black-market products using liquid chromatography–tandem mass spectrometry; Benjamín Velasco-Bejarano, Jahir Bautista, Ma. Olivia Noguez, Evangelina Camacho, Martha E. Rodríguez and Leonardo Rodríguez, *Drug Test. Analysis* (**2017**) DOI 10.1002/dta.2294





ELIMINATION STUDY
CONSUMPTION OF MEAT CONTAMINATED WITH CLENBUTEROL

This study was determined the pharmacokinetics of clenbuterol administered through single or repeated exposure to meat contaminated with clenbuterol.

This study was conducted in healthy volunteers.

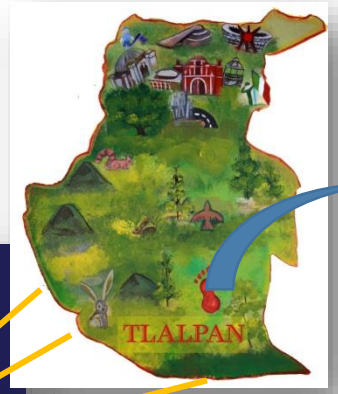






Laboratory Site

Comisión Nacional de Cultura Física y Deporte



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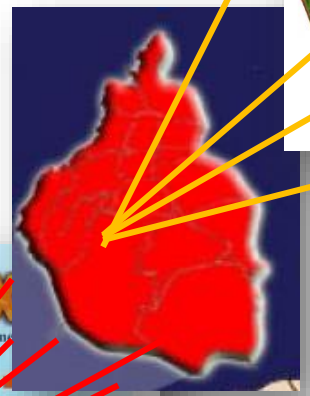


Laboratorio Nacional de Prevención y Control del Dopaje





Design of the study

Site of the experiment and urine sample colection: CENAR, Mexico

Males
- ✓ 20 males
- ✓ 20-40 years old
- ✓ No athletes
- ✓ Healthy
- ✓ Free clenbuterol

Females
- ✓ 20 males
- ✓ 20-40 years old
- ✓ No athletes
- ✓ Healthy
- ✓ Free clenbuterol
- ✓ Participants should record the stage of their menstrual cycle and/or whether they are taking contraceptives

Site of sample analysis: Anti-doping Laboratory Mexico

CNAR: National Center of High Performance by his initial in Spanish

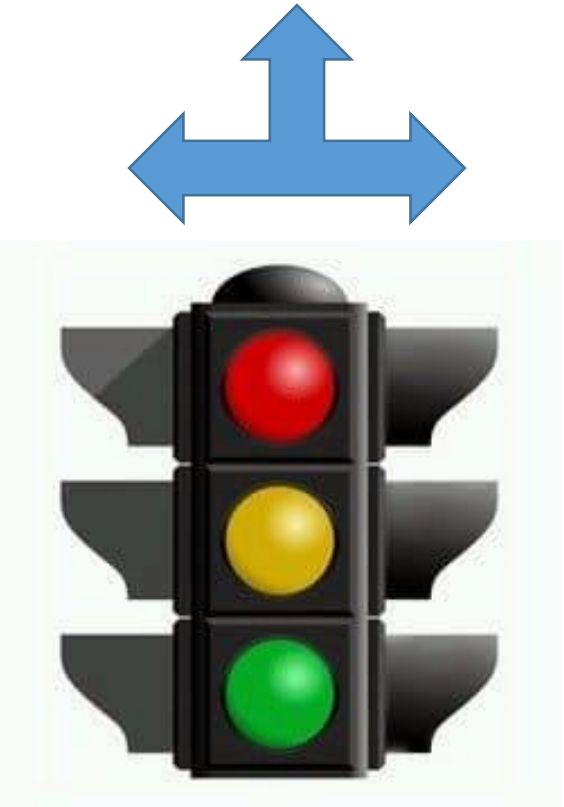




Inclusion / Exclusion criteria

Inclusion:

- ✓ Healthy;
- ✓ 20-40 years of age;
- ✓ Before enrolment, all participants will receive a physical examination that includes a medical history;
- ✓ Volunteers will read the letter of information to participants regarding the experiment;
- ✓ Sign an informed consent to participate in the study.



Exclusion:

- ✓ Participants with a history of cardiac troubles; neurologic, digestive and or other diseases will be excluded from the study.
- ✓ Participants taking nutritional supplements or medication one week before, during and until the last sample collection (contraceptive in female OK).
- ✓ Elite athletes participating in national or international competitions.





The samples of bovine meat used in the study were bought in places (market and butcher shops) that were suspicious of clenbuterol contamination in Mexico City

Mexico City Map

This values would prevent any potential risk of toxic effects associated with the ingestion of very high doses of clenbuterol.

The concentration of clenbuterol in meat was 1-10 mg/kg this values are similar to the cattle treated with anabolic doses of clenbuterol.

The clenbuterol was contained in meat obtained from cattle illegally treated with this drug in Mexico.

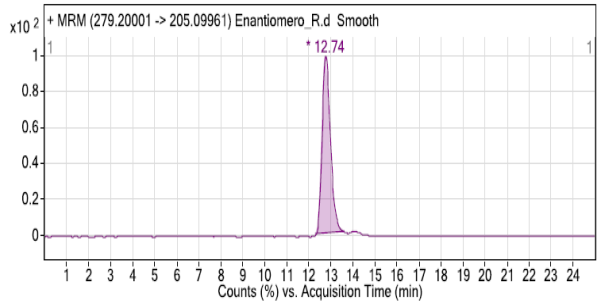
The methodology used in this study is the following

In Mexico is forbidden administered contaminated meat of intentionally manner to a volunteer in order to research

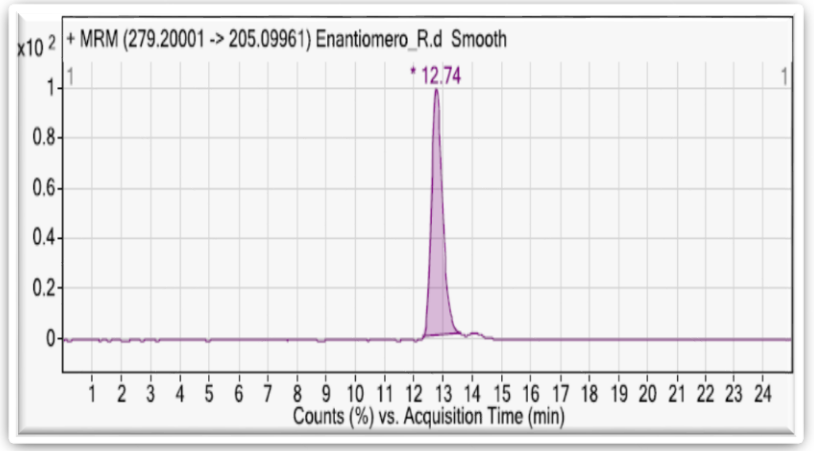
The methodology used in this study is the following

The meat is contaminated with clenbuterol

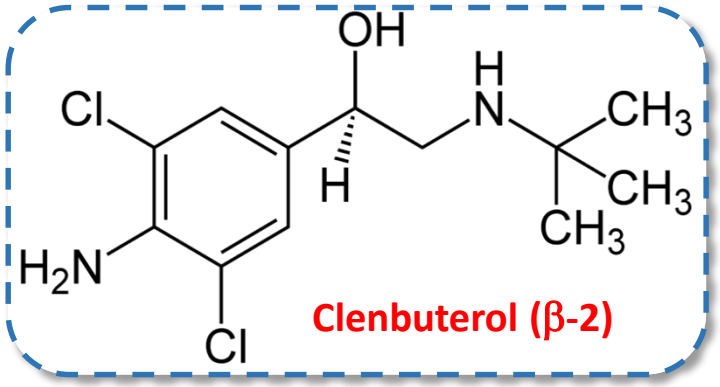
250 g



38 Different batches of bovine meat



Clenbuterol concentration







Single exposure: Each volunteer

10 volunteers

only one meal was consumed

10 volunteers

- ✓ 250 g of bovine meat was administered to a healthy volunteer, without restriction of consumption of food (without bovine meat, Chicken and Pork) and water.
- ✓ All volunteers were under medical supervision (Clinical facilities) pre and post ingestion of meat.
- ✓ The morning of the experiment and before of the ingestion, the volunteer collected a urine sample (basal levels)





Repeat exposure: Each volunteer.

10 volunteers

10 volunteers

- ✓ 3 portions of 250 g meat was administered to a healthy volunteer (Three consecutive meals) without restriction of consumption of food (without bovine meat, Chicken and Pork) and water.
- ✓ All volunteers were under medical supervision (Clinical facilities) pre and post ingestion of meat.

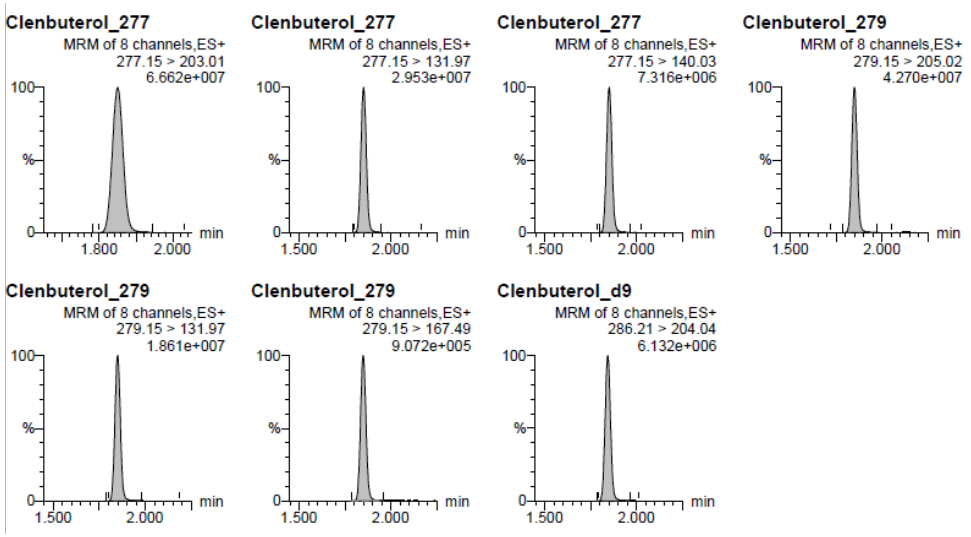




Sample collection and Analysis

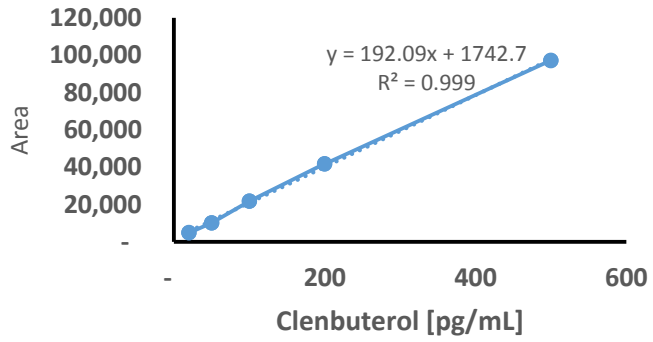
- Each volunteer collected urine samples starting with a sample before ingestion T=0 to establish the baseline, followed by the collection of every mictions.
- The follow samples were collect during the study period. All samples were collected individually (no pooled, different urine samples, different code)
- The samples were collected, indicating the time of collection, volume of miction.
- The samples were transported to the Anti-doping Lab Mexico City for their analysis.
- HPLC-MS/MS

Detection



Cuantification

Calibration Curve

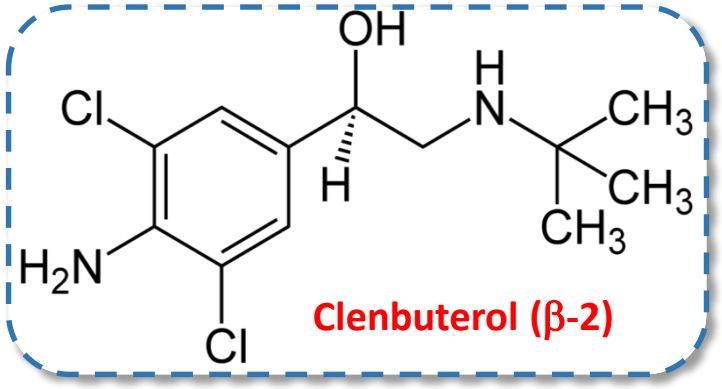


Concentration levels 20-500 pg/mL

LC MS/MS; XEVO, WATERS,

LOD= 10 pg/mL

LOQ= 20 pg/mL ; u_c=10%



Clenbuterol (β-2)

Single administration: Each volunteer

10 volunteers

10 volunteers

- ✓ A dose of 20 mcg was administered to a healthy volunteer, without restriction of consumption of food (without bovine meat) and water.
- ✓ All volunteers were under medical supervision (Clinical facilities) pre and post administration of pills.
- ✓ The morning of the experiment and before of the ingestion, the volunteer collected a urine sample (basal levels)
- ✓ Each volunteer collected urine samples starting with a sample before administration T=0. The samples was be kept 2-8 °C.





Results

Simple Exposition

Repeat Exposition

Finally 22 males and 22 females voluntieers paricipated in the study

1960 Urines samples were analyzed

Simple administration

10 males and 10 females voluntieers paricipated in the study

960 Urines samples were analyzed

Staff members of the Doping Lab Mexico





THIS PROJECT WAS FUNDED BY FEDERAL INSTITUTIONS OF MEXICO





Thank you!!