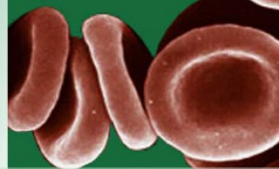




IDA Dresden



Hair Testing

...to provide additional evidence in doping cases
of potential (clenbuterol) contaminations

Detlef Thieme

Institute of Doping Analysis, Dresden, Germany



[M. Jahn (www.kreischa.net)]

0. Principles of **Hair Testing**

1. **Cases**: Long-Term Detection of Drugs

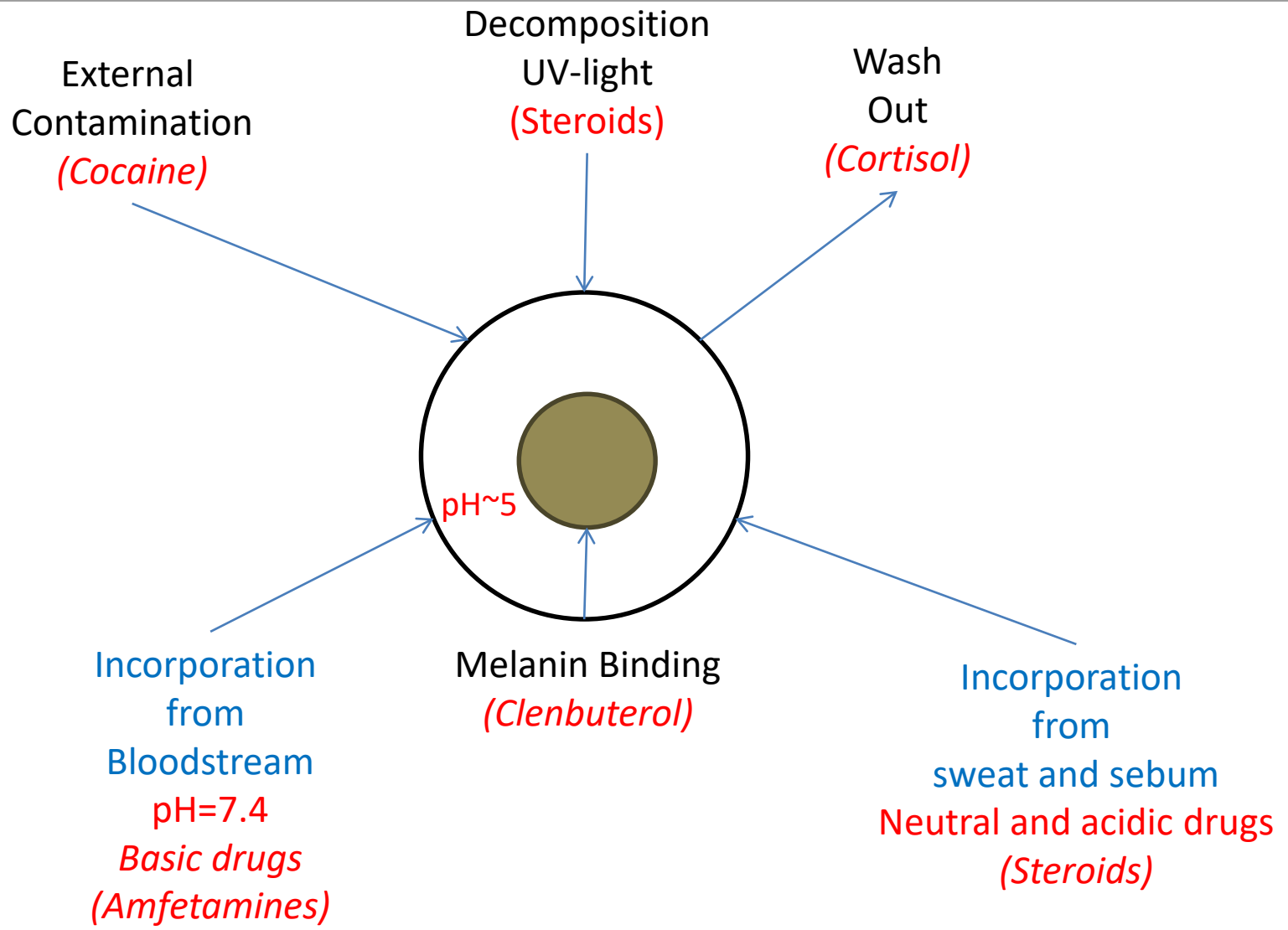
2. Clenbuterol: **Field Study** in Mexico

3. Clenbuterol WADA Project: **Administration Study**

4. Clenbuterol: Real **Doping Cases**

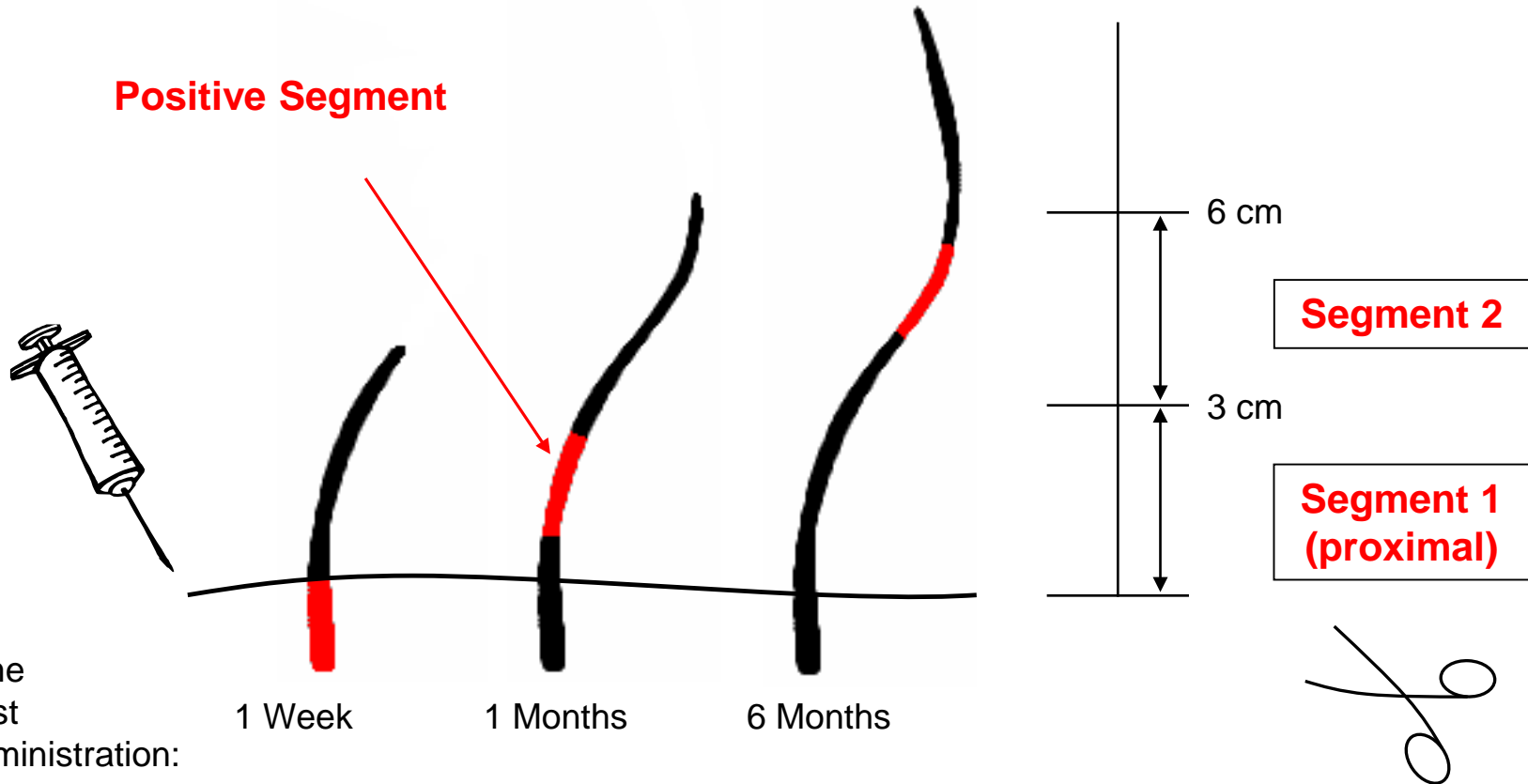


0. Principles of Hair Incorporation





0. Segmental Analysis

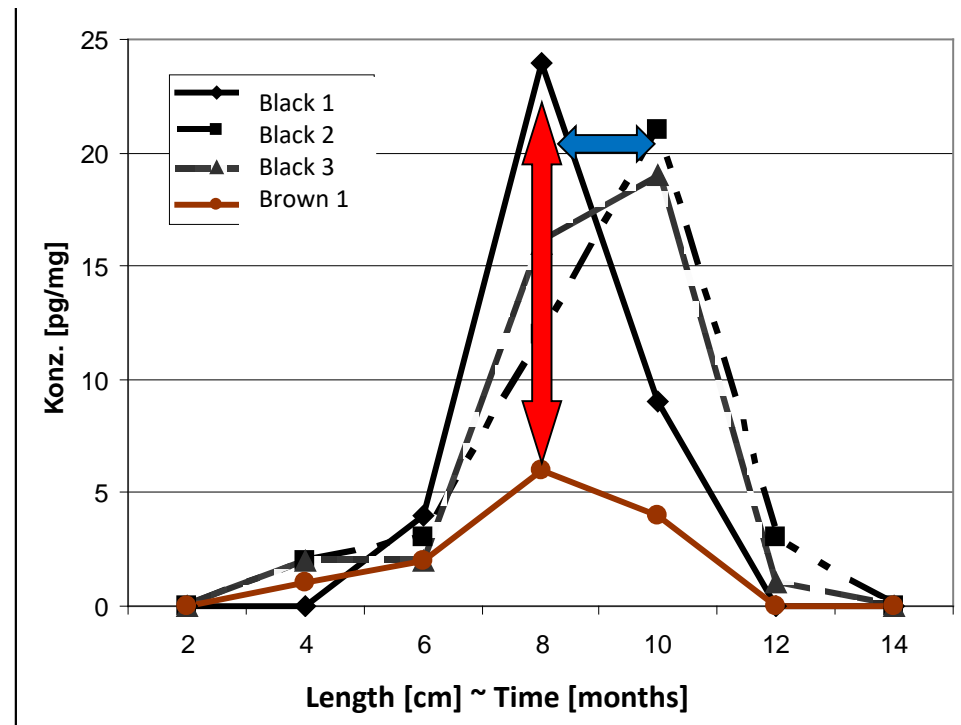




0. Clenbuterol-Application – 4 Horses

Dose: 2x 0,8µg/kg KG/d, oral (10 Days)

Hair collected 4 months post administration

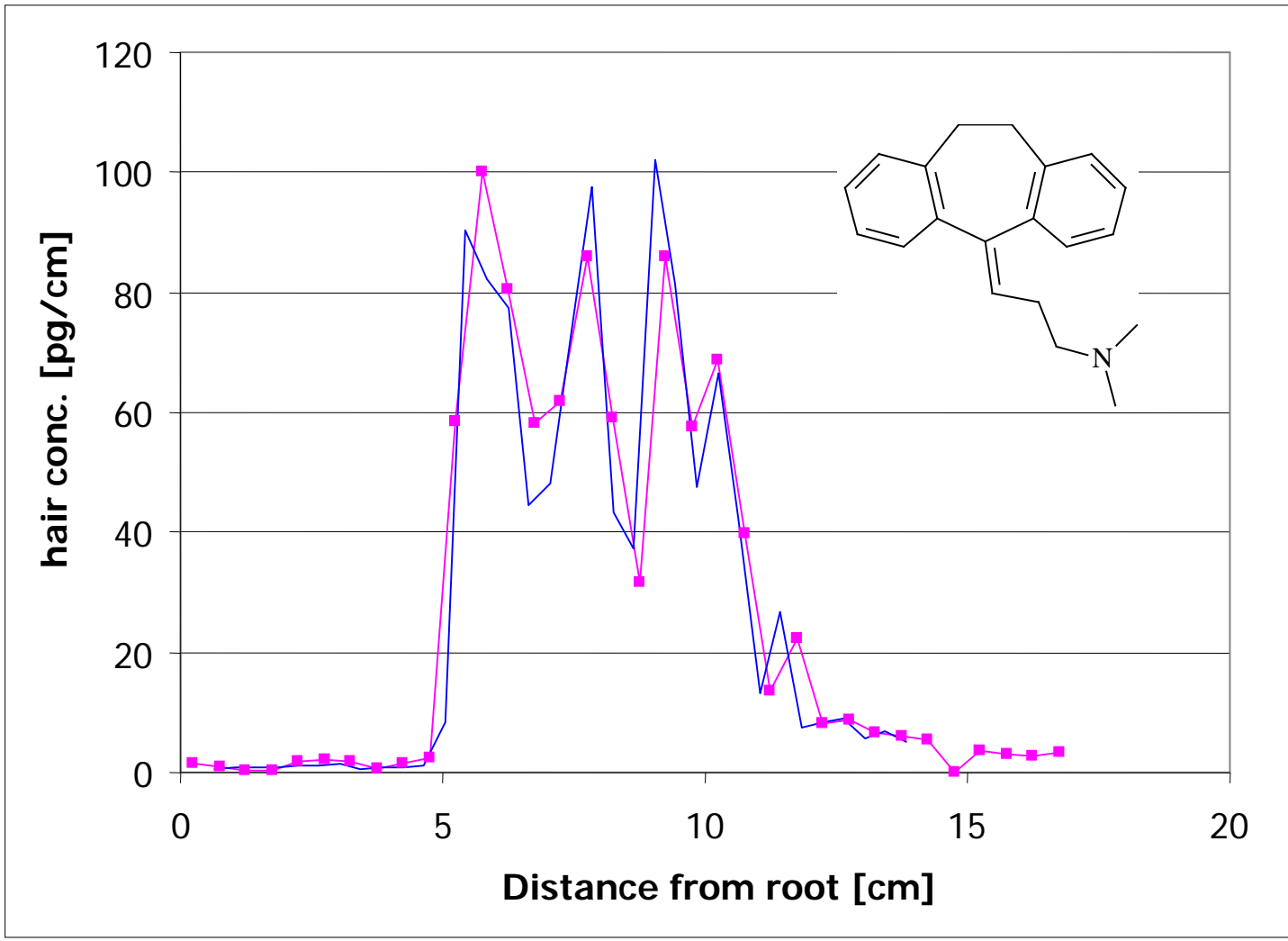


→ Individual Growth Rate

→ Hair Color



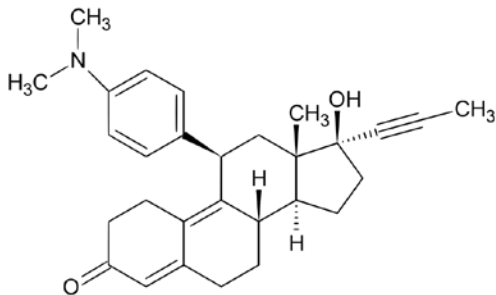
1. Amitriptyline Case (~40 children)





1. Single administration of Mifegyne®

- Progesterone receptor antagonist
- Used for early terminating of pregnancy





1. Single administration of Mifegyne®

<http://www.womenonwaves.org/en/page/702/how-to-do-an-abortion-with-pills--misoprostol--cytotec>

HOW TO GET MISOPROSTOL

In some countries women can get Misoprostol at their local pharmacies and **use it alone**.

...

Sometimes it is sold **over the counter** without a prescription, or sometimes a prescription is required

...

A woman can of course also **make her own prescription** (see example)

name doctor:
Registration number:
Address:

Date:

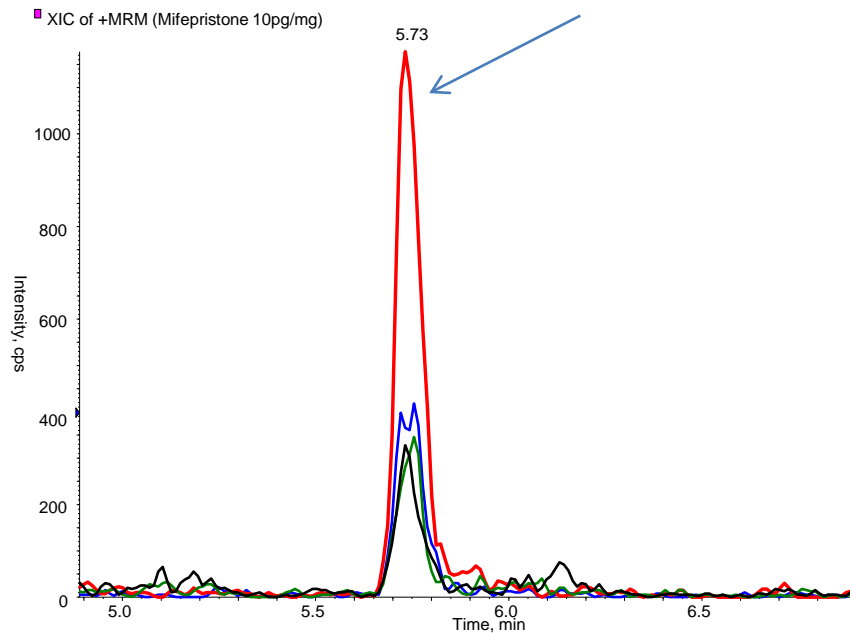
R/ Misoprostol 200 mcg
Dtd: 12 tablets
S: use known

(signature doctor)

name patient:
date of birth

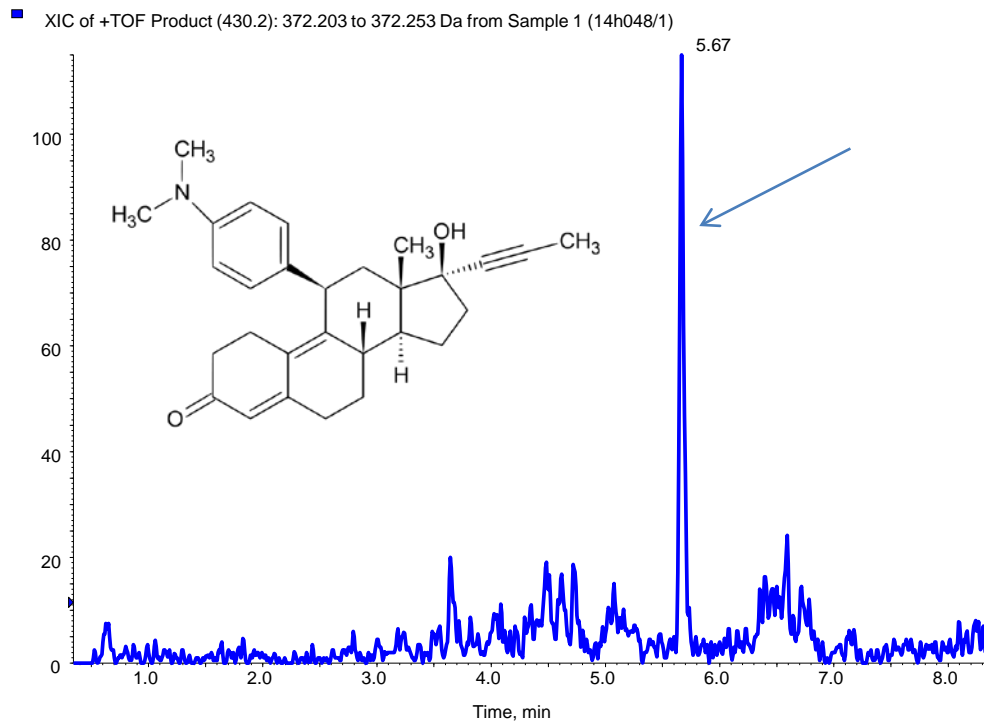
1. Single administration of Mifegyne®

Single Administration of 200mg Mifepristone
Could be Detected in Hairs of Two Victims



by LC-MS/MS

or LC-MS/HRMS





96 Hair Tests in Bodybuilding

	Compound	Hair Conc (pg/mg)	Urine
29 y.o. Male bodybuilder bleached hair 0-3 cm ~160 doping agents confiscated	Clenbuterol	2	n.d.
	Boldenone	3	n.d.
	Boldenone Undecylenate	400	
	Drostanolone	4	Drostanolone
	Drostanolone Enantate	30	
	Metandienone	220	Metandienone-Mb
	Nandrolone		
	Phenylpropionate	145	Norandrosterone
	Stanozolol	43	OH-Stano
	Testosterone Enantate	500	
	Trenbolone	15	Epi-Trenbolone
	Trenbolone Acetate	10	
	Trenbolone Enantate	270	
	Tamoxifen	150	n.d.
	Anastrozol	85	Anastrozol
	Testosterone	500	T/E
Fluoxymesterone	n.d.	Mb.	

Findings in Hairs of Presumptive Steroid Users

Anabolic Steroid	No of cases	
	96 Hair Samples	Confiscations
Testosterone Enantate	29	561
Metandienone	24	206
Testosterone Propionate	24	558
Clenbuterol	8	59
Trenbolone Acetate	8	174
Stanozolol	7	116
Trenbolone Enantate	7	194
Drostanolone Propionate	4	35
Boldenone Undecylenate	3	125
Nandrolone Decanoate	3	134
Testosterone Decanoate	3	64
Testosterone Phenylpropionate	3	57
Drostanolone Enantate	2	45
Nandrolone Phenylpropionate	1	36
Testosterone Cypionate	1	81

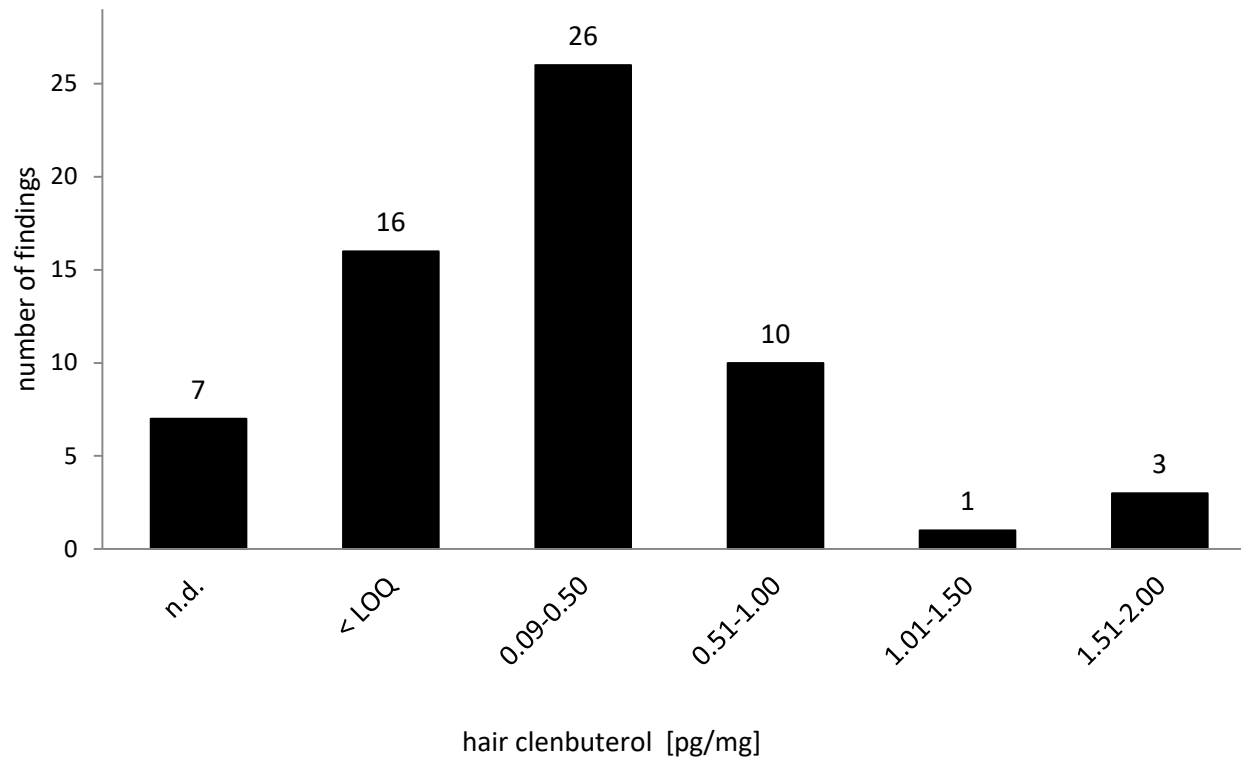
Correlation between Urine and Hair (96 Cases)

		Hair (214 findings)	
		pos	neg
Urine	pos	56.5 %	27.1 %
	neg	16.4 %	(9 cases)

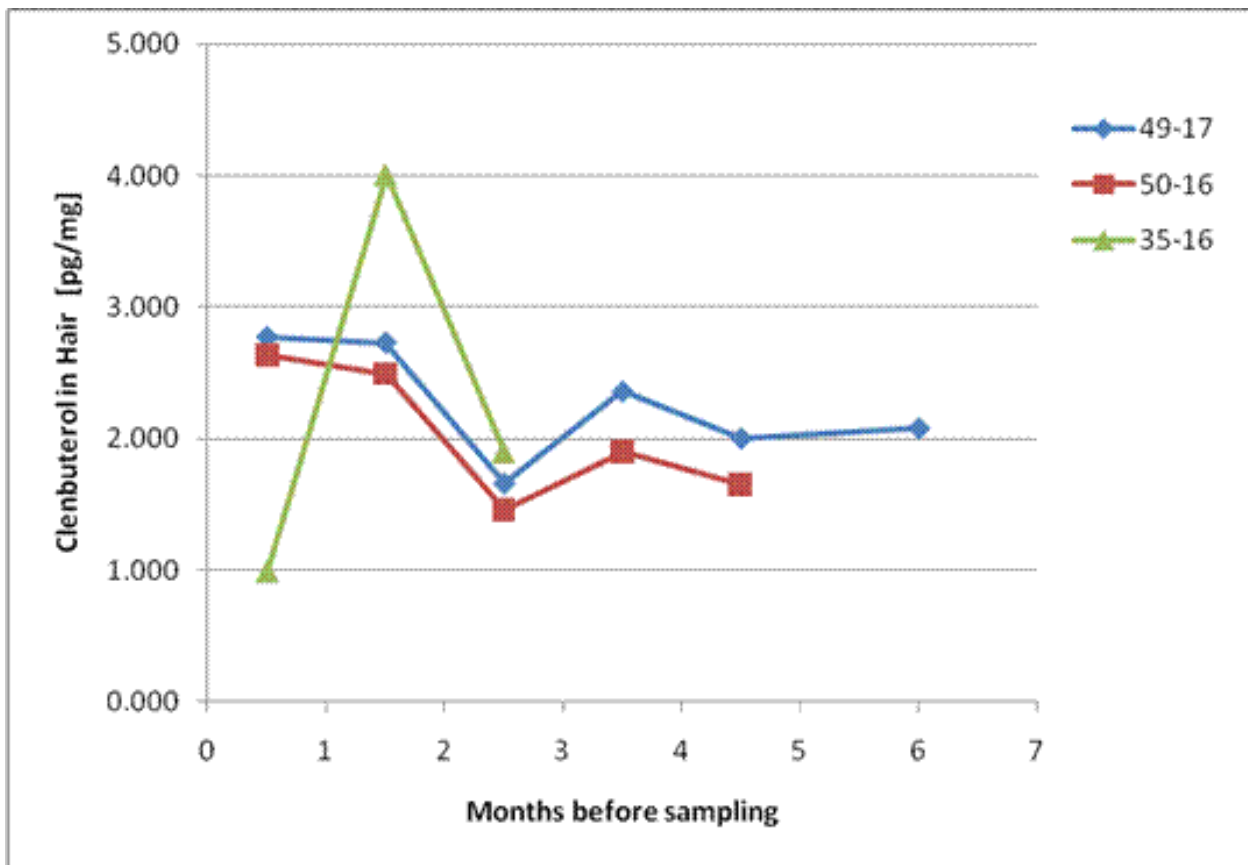
2. Field Study Mexico (FIFA)

Team- Classification	Samples collected [n]	positive findings (%)	Average conc. [pg/mg]	Max [pg/mg]
U20	19	18 (94.7)	0.18	1.29
U17	24	20 (83.3)	0.37	1.79
local	20	18 (90.0)	0.49	1.90

2. Clenbuterol Hair Concentrations



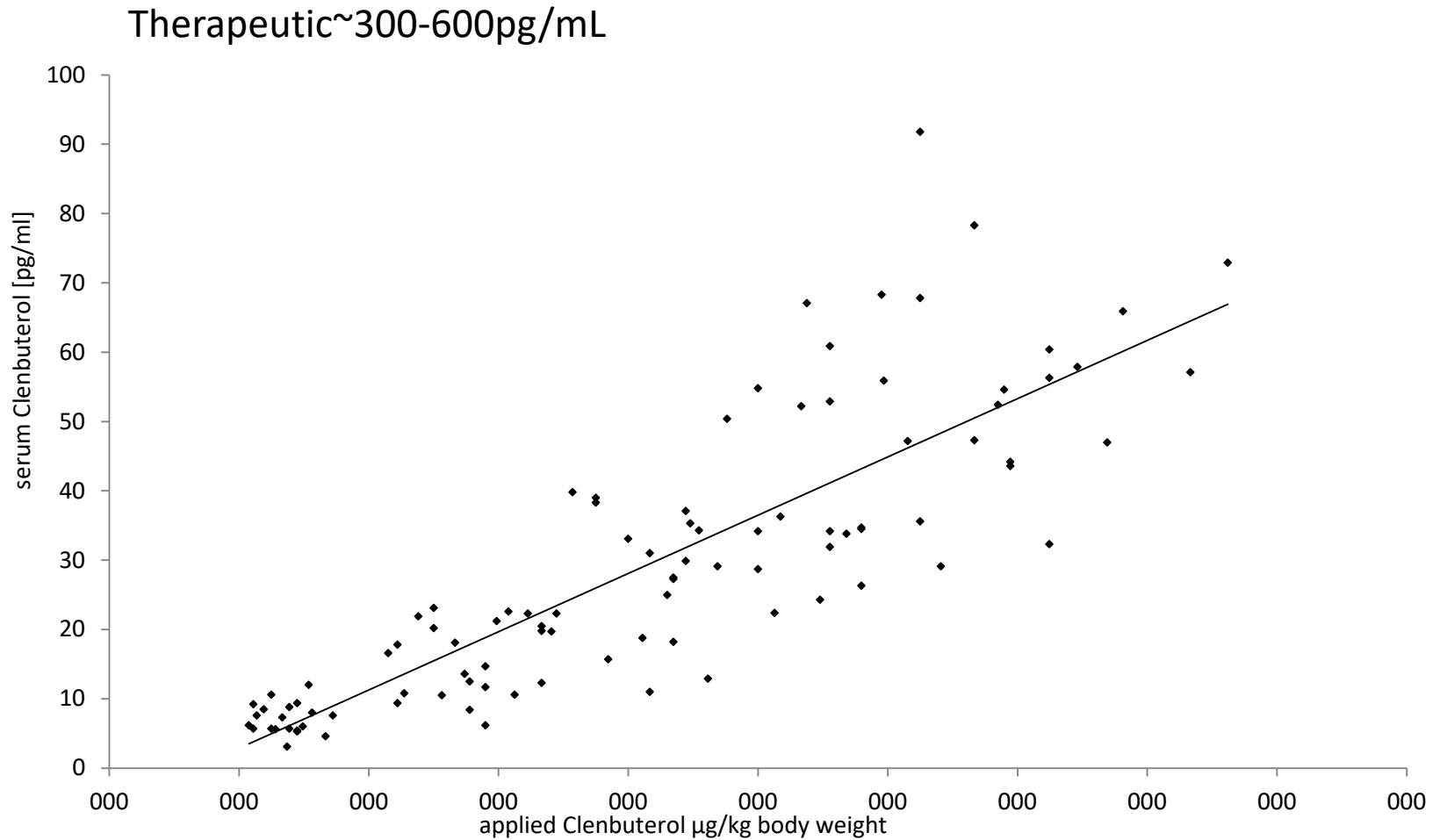
2. Field Study Mexico



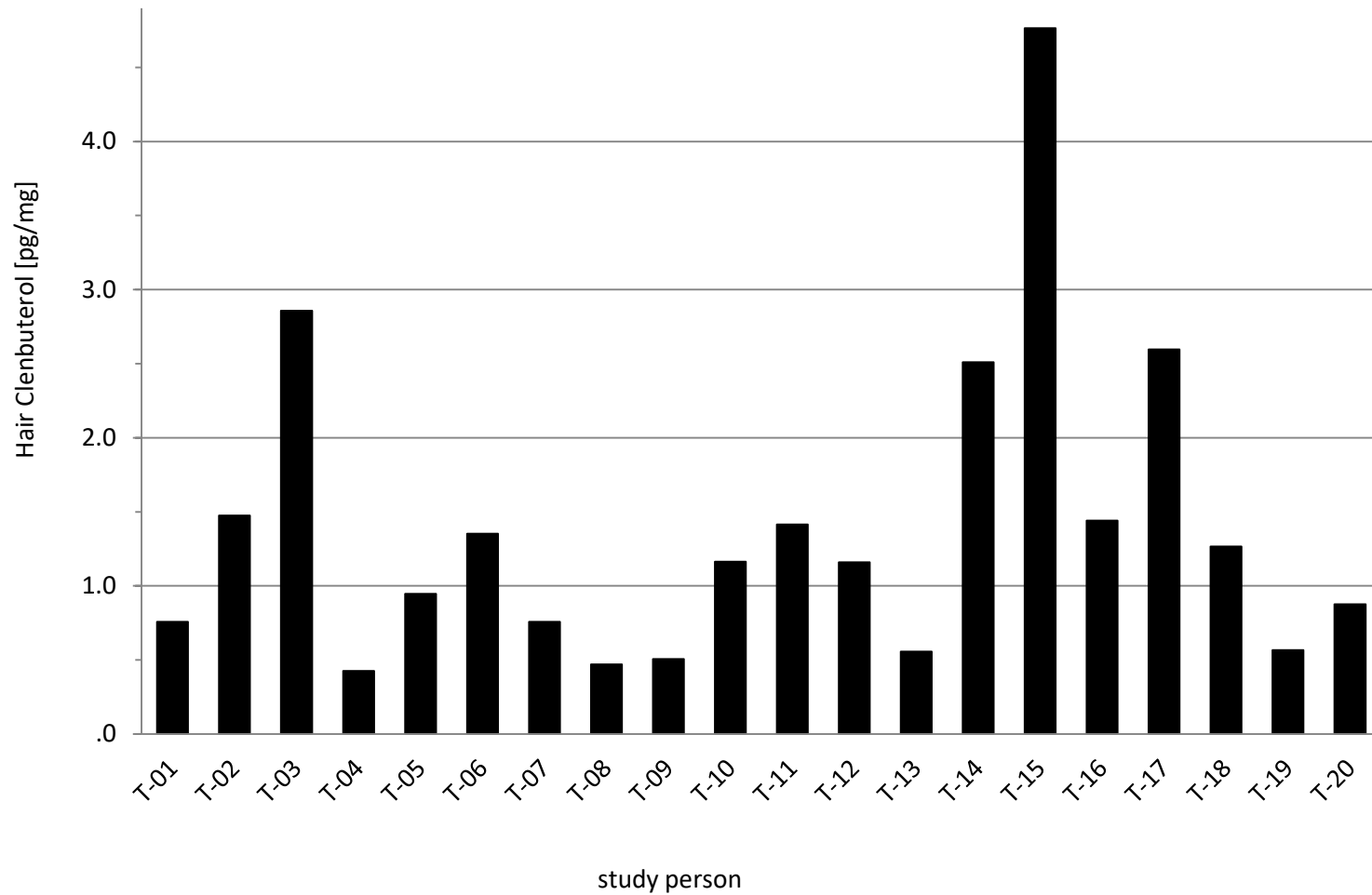
3. Administration Study (WADA)

ADI (JECFA, 1998)	0.004 $\mu\text{g}/\text{kg}/\text{d}$
Admin Study (Average)	0.02 $\mu\text{g}/\text{kg}/\text{d}$
Therapy	0.4 $\mu\text{g}/\text{kg}/\text{d}$
Abuse (?)	1-2 $\mu\text{g}/\text{kg}/\text{d}$

3. Dosage vs. Serum Level



3. Clenbuterol Hair Concentrations



4. Clenbuterol Cases (before 2012, LOD=0.9 pg/mg)

Code	Discipline	Urine	Hair	Hair Concentration
12D002	Cycling	02.09.11 50pg/ml	04.01.12 Head Hair (dark brown);	0-2,5cm: neg 2,5-5cm: neg 5-7,5cm: neg
10D171	Table Tennis	?	06.10.10 Head Hair (brown);	0-2cm: neg 2-5cm: neg
10D151	Table Tennis	?	24.09.10 Head Hair (dark blond);	0-2cm: neg 2-4cm: neg

4. Clenbuterol Cases (after 2012, LOD=0.02 pg/mg)

Code	Discipline	Urine	Hair	Hair Concentration
14h082	Cycling	?	01.12.14 Head Hair (dark brown);	0-1,5cm: neg 1,5-4cm: neg
14h026	(FIFA, Guatemala)	?	22.04.14 Head Hair (dark brown);	0-2cm: trace
14h027	(FIFA, Guatemala)	?	22.04.14 Head Hair (dark brown);	0-3cm: 0,5pg/mg
13h0039	(FIFA, Guatemala)	?	29.03.13 Head Hair (black);	0-0,5cm: neg
13h0040	(FIFA, Guatemala)	?	29.03.13 Head Hair (dark brown);	0-3cm: neg
13h0041	(FIFA, Guatemala)	?	29.03.13 Head Hair (dark brown);	0-5cm: neg
13h0042	(FIFA, Guatemala)	?	29.03.13 Head Hair (dark brown);	0-3cm: 0,03pg/mg
13h0043	(FIFA, Guatemala)	?	29.03.13 Head Hair (black);	0-3cm: 0,06pg/mg; 3-6cm: 0,06pg/mg; 6-9cm: 0,08pg/mg



4 Cases Track Cycling

1. Female

Urine (OOC, 11.12.13): Clenbuterol 0,02 ng/ml
Hair (22.01.14): Head Hair, brown
0-2 cm / 2-4 cm / 4-6 cm
all segments negative

2. Male

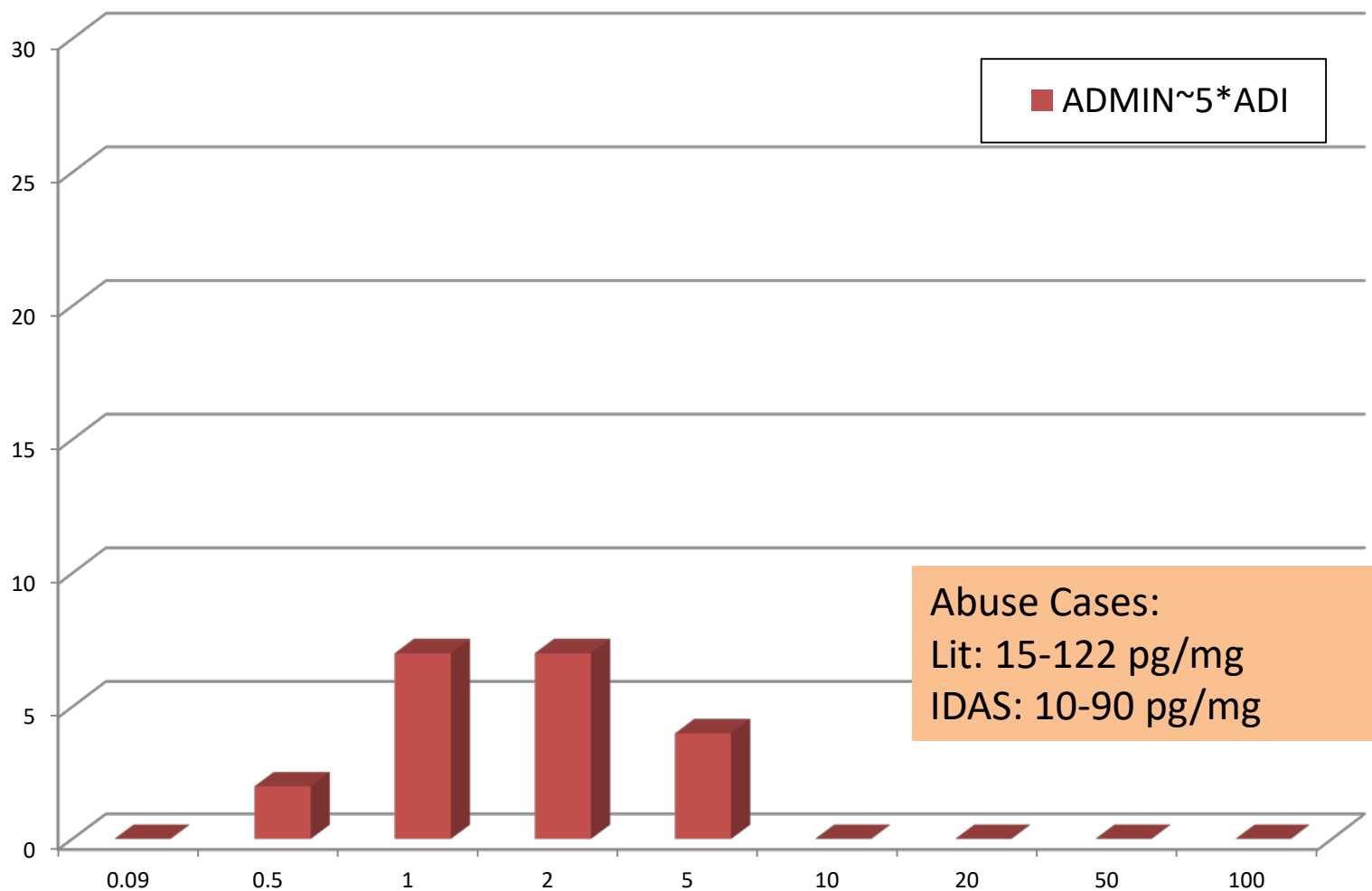
Urine (OOC, 12.12.13): Clenbuterol 0,08 ng/ml
Hair (22.01.14) Head-hair, dark brown
0-2 cm: ***0,08 pg/mg Clenbuterol***
(November 2013 – January 2014)
2-4 cm: ***negative***

3. Male

Urine (23.01.14): Clenbuterol 0,13 ng/ml
Hair (17.03.14): Pubic Hair, dark blond
0-2 cm: ***negative***

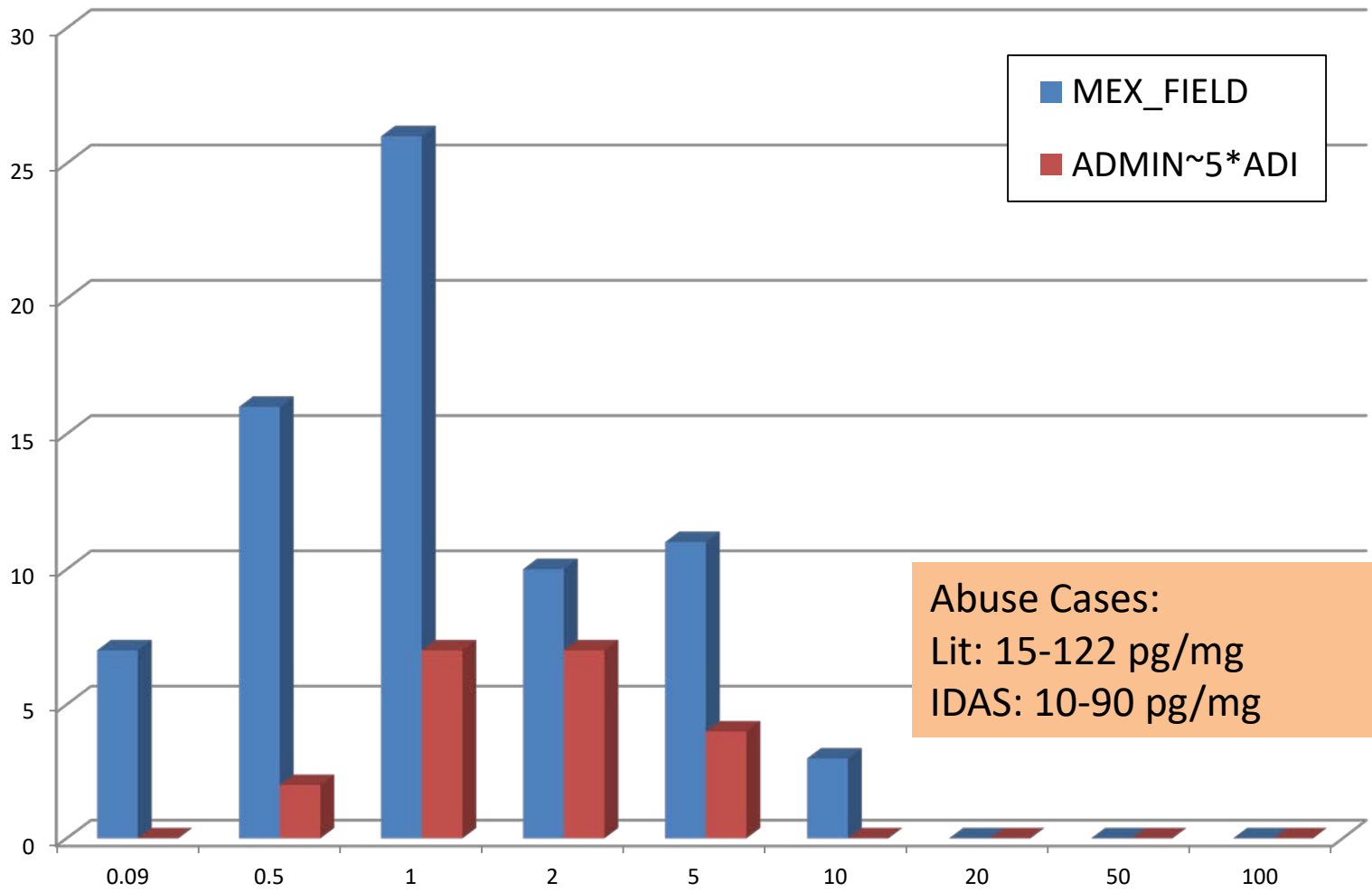


4. Discrimination Power of Hair Testing for Clenbuterol

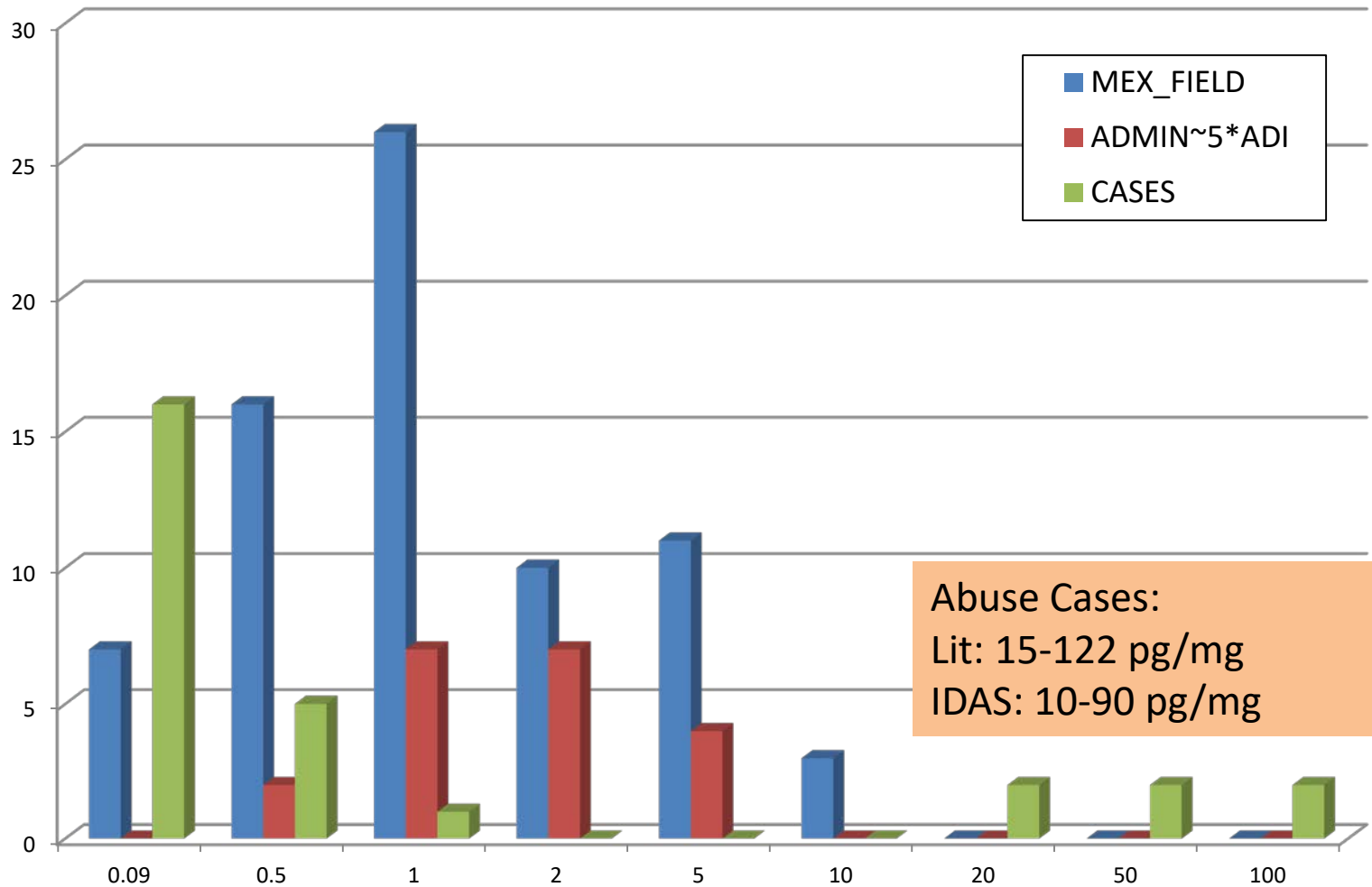




4. Discrimination Power of Hair Testing for Clenbuterol



4. Discrimination Power of Hair Testing for Clenbuterol



4. Clenbuterol Cases

Code	Discipline	Urine	Hair	Hair Concentration
15h001	Weightlifting (WM Women)	13.11.14 38 pg/ml	10.01.15 Head Hair (brown, colorized);	0-2cm: 20pg/mg; 2-4cm: 18pg/mg
15h002	Weightlifting	10.11.14 50 pg/ml	10.01.15 Head Hair (dark brown);	0-2cm: 23pg/mg; 2-4cm: 18pg/mg
15h003	Weightlifting	09.11.14 100 pg/ml	10.01.15 Head Hair (black);	0-2cm: 86pg/mg; 2-4cm: 81pg/mg

Hair Testing potentially suitable to confirm Clenbuterol Doping at low urinary concentrations!!!



Case report: Possession of...

- Testosterone-enantate, Testosteronepropionate, Testosteronecypionate, Testosteroneisocaproate, Testosteronephenylpropionate, Testosterone decanoate
- Trenboloneacetate, Trenbolone-enantate, Trenbolonehexahydrobenzoate
- Metenolone-enantate,
- Nandrolonedecanoate,
- Boldenoneundecylenate,
- Oxandrolone,
- Metandienone,
- Oxymetholone,
- Fluoxymesterone,
- Methyltestosterone
- Stanozolol
- Tamoxifen
- **Clenbuterol**
- Somatropin, GH-Fragment 176-191, mod- GRF



Urine Test

- Elevated TE (microbial degradation)
- LH < 0,1 mIU/ml;
- Norandrosterone, Noretiocholanolone (high)
- Drostanolone (metabolite, low)



Hair Test

- *Boldenone* (6 pg/mg),
- *Clenbuterol* (6 pg/mg)
- *Metandienone* (9 pg/mg),
- *Nandrolone* (7 pg/mg),
- *Testosteron-Propionate* (7 pg/mg)
- Testosterone 6 pg/mg > normal

Summary

- Hair Testing lacks sensitivity but gains retrospection.
Follow up possible weeks after urine test (if hair is available)
- (Dark) hair concentration of clenbuterol are comparatively well dose related.
- High Discrimination Power between Low ($5 \times \text{ADI}$) and Hi (Abuse) cases.
- A proposed threshold of 1...5 pg/mg clenbuterol in hair could contribute to discriminate most of the pending clenbuterol cases.
- Increasing reporting levels of clenbuterol would cause potential disregarding real (even excessive) cases.
- Estimated Clenbuterol Contamination Dosages in high risk areas (Mexico) correspond to administrations in the order of magnitude of $2 \times \text{ADI}$.

Acknowledgements

- Administration Study was funded by WADA
- Field Study funded by FIFA
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- Administration study was accomplished by Lena Gfrerer at the Institute of Legal Medicine (Munich University)
- Analytical work was carried out by Aniko Krumbholz and Patricia Anielski (IDAS Dresden).