



Study of chlordecone elimination in ewe

Development of a LC-MS/MS method and a QuEChERS extraction method to analyze CLD and its metabolites in urine and in feces

47th congress of GFP (Groupe Français des Pesticides)

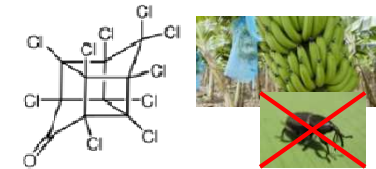
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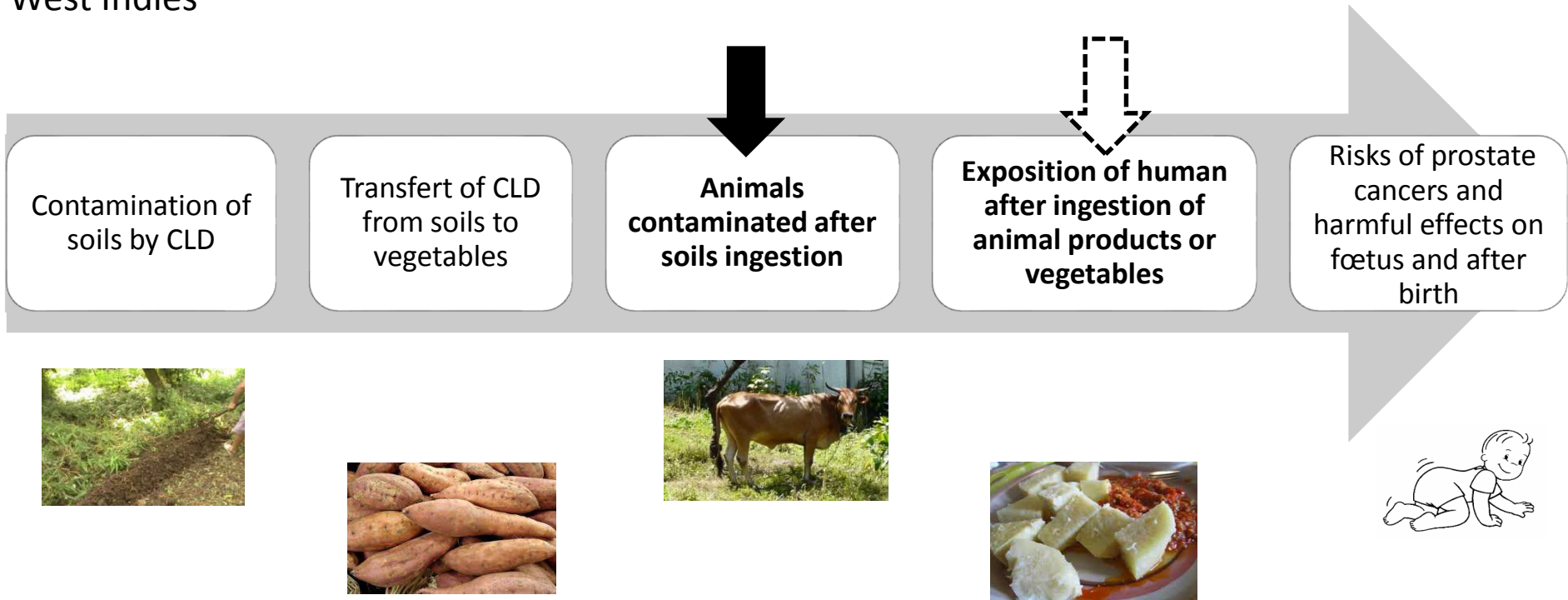
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Chlordecone: A persistent molecule in West Indies soils



Organochlorine pesticide intensively used against banana weevil from 1960 to 1992 in the West Indies

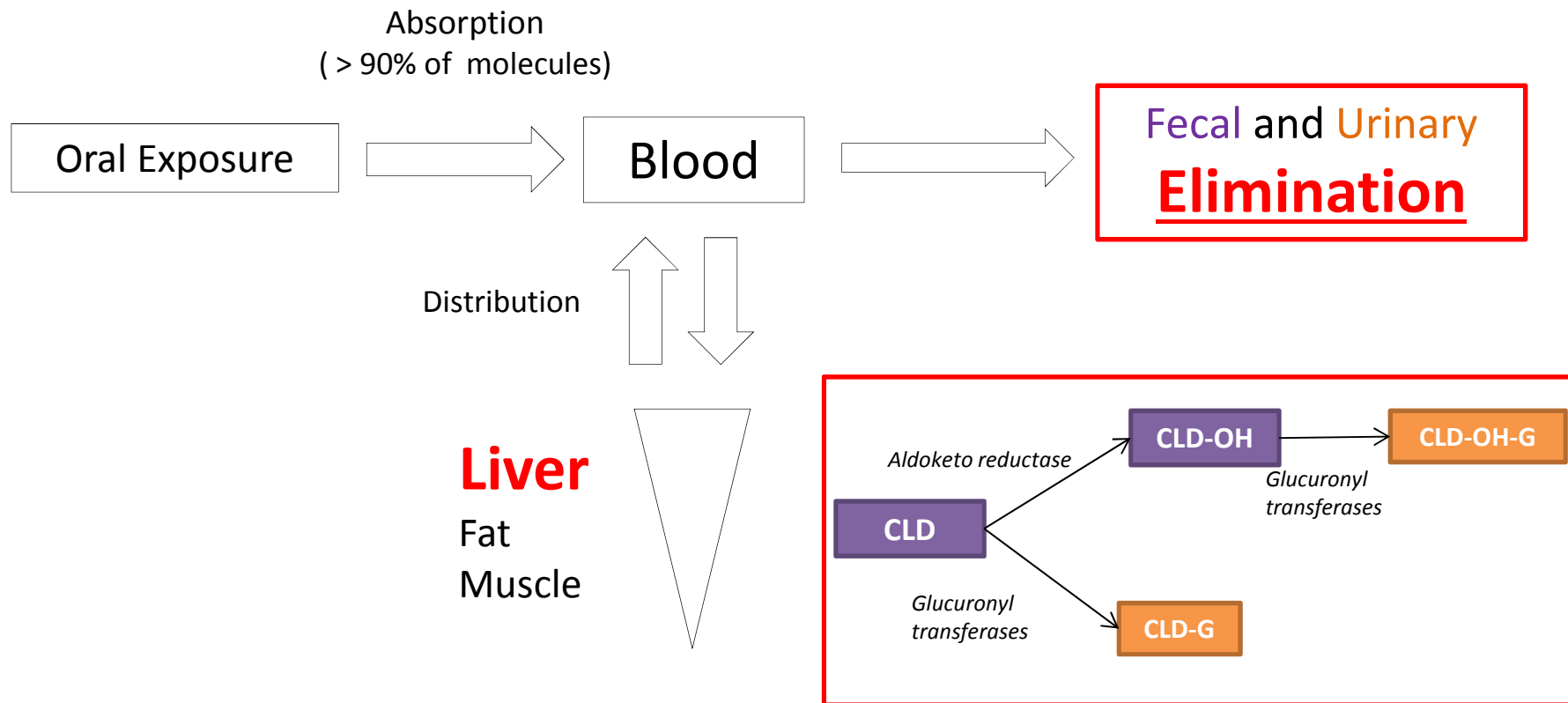


→ It is important to propose depuration strategies for contaminated animals

How CLD is eliminated from animals bodies ?

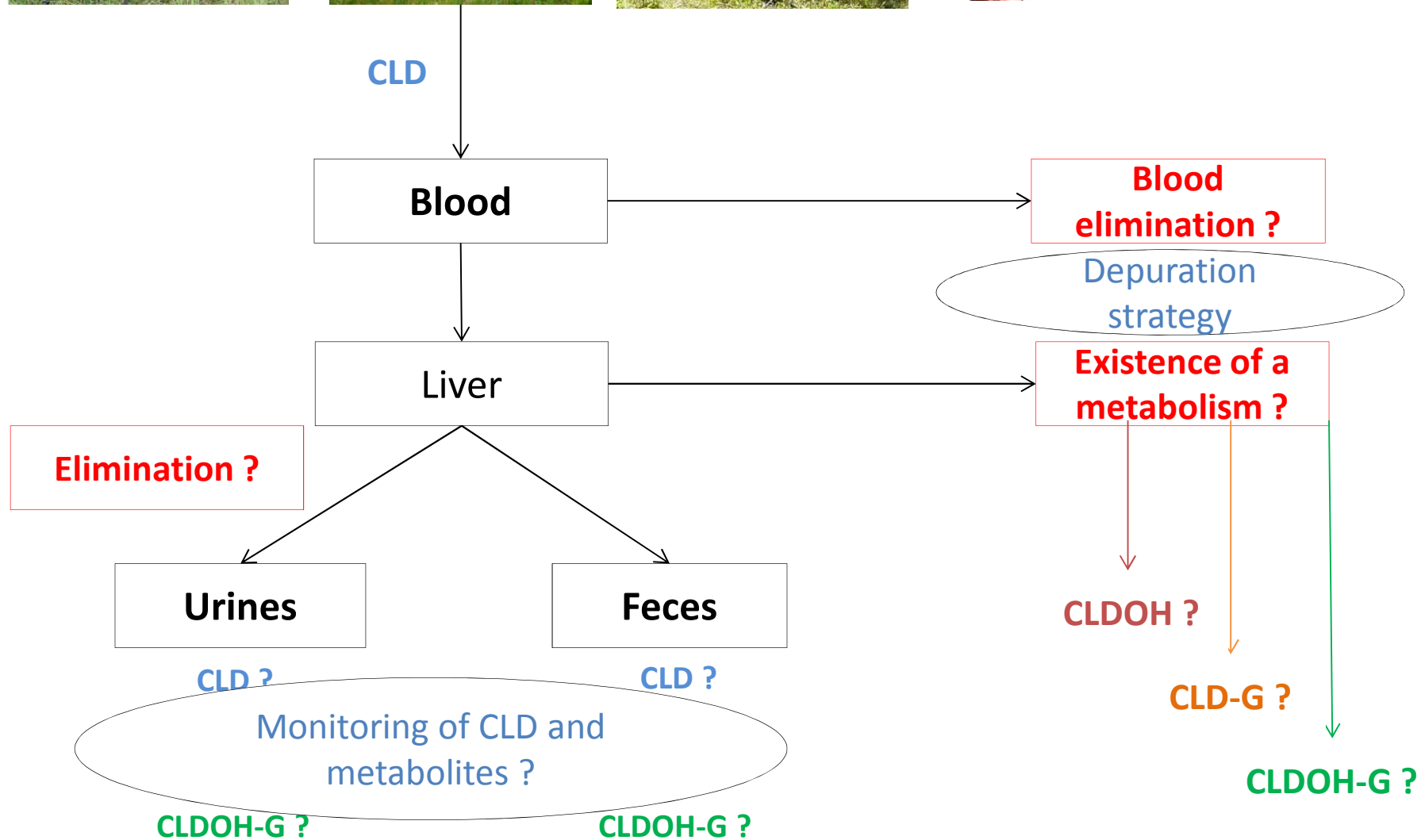
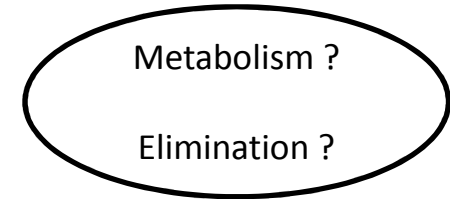
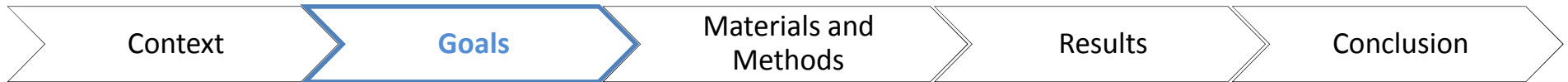


Toxicokinetic of chlordecone in animals



Established metabolism for human, pig and gerbil

CLD: Chlordecone
 CLD-OH: Chlordecol
 CLD-OH-G : Conjugated chlordecol
 CLD-G: Conjugated chlordecone

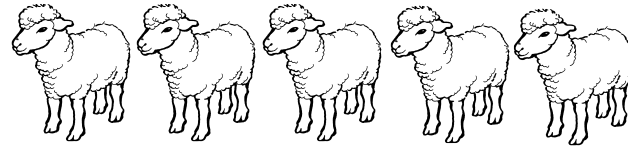


Characterisation of the CLD elimination in ewe



Design of the toxicokinetic study in ewe

Dose
1 mg/kg BW



Foley probe

Diaper

ADAPTATION	EXPOSURE	DECONTAMINATION
14 days	Injection of a unique IV dose of CLD	84 days



Sampling during decontamination of

Blood → Serum → CLD analysis by the CART

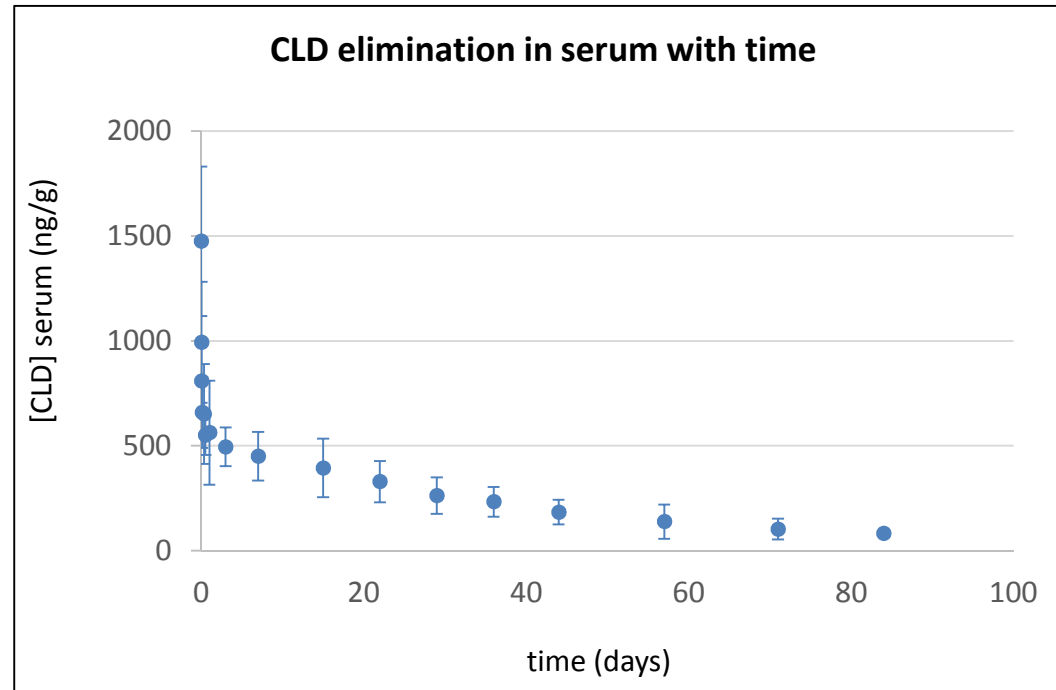
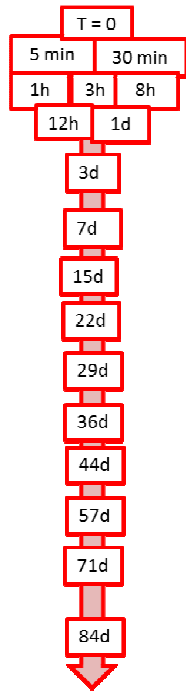
Urines and Feces → No analytical methods to analyze CLD and its metabolites



Syringe

Foley probe

Serum elimination of CLD in ewe



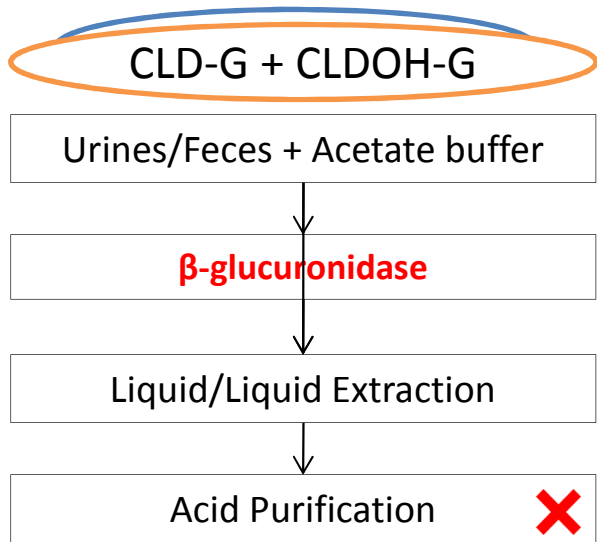
- ✓ $T_{1/2}$ mean for ewe is 28.5 ± 3.9 days
- ✓ Provides information for the animal decontamination

What about in urine and feces and in which forms ?



Development of analyticals methods to analyze CLD and CLD's metabolites in urine and in feces

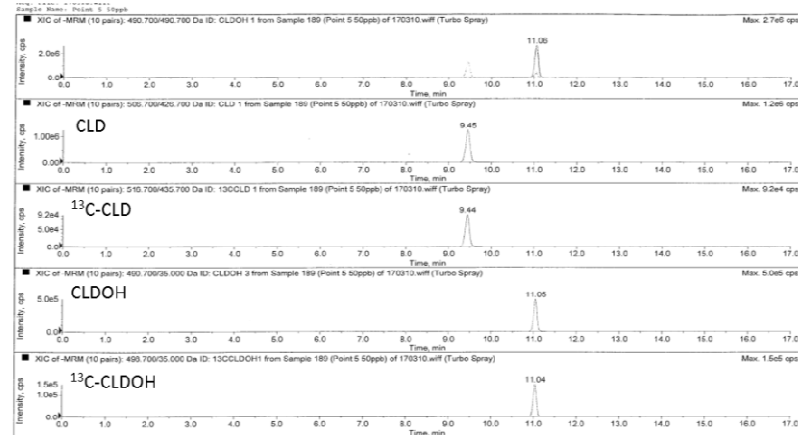
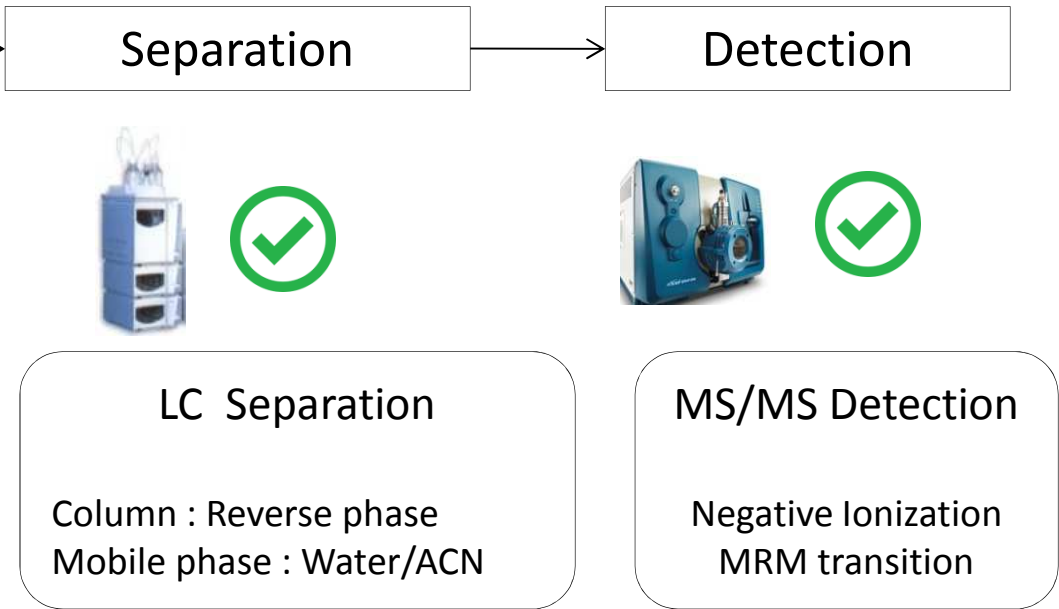




Chung et al. 2011



→ Development of a QuEChERS extraction with isotopic dilution



Classic QuEChERS

(Quick, Effective, Cheap, Easy, Rugged and Safe)

Anastassiades et al. 2003

*Extraction +
Salting-out*

*dSPE
Purification*

10 g of matrix

Addition of water if there is less than 80% of water in the matrix

Extraction with 10 mL of ACN -Stirring 1 min

Salts Addition of 4 g MgSO₄ + 1 g NaCl
(Possible to use buffer)- Stirring 30 s

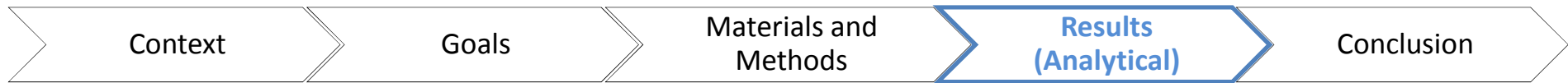
Centrifugation 5 min > 3000 rpm

Add the supernant to the dSPE sorbent

Stirring 30 s

Centrifugation 5 min > 3000 rpm

Injection of the purified extract

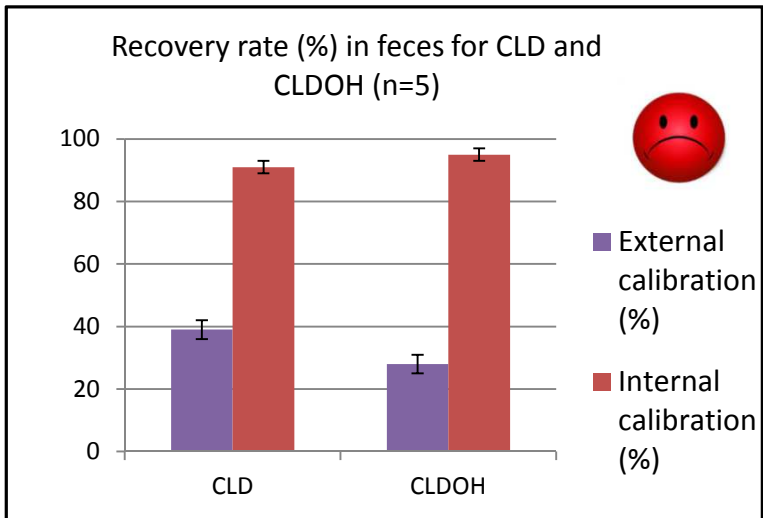
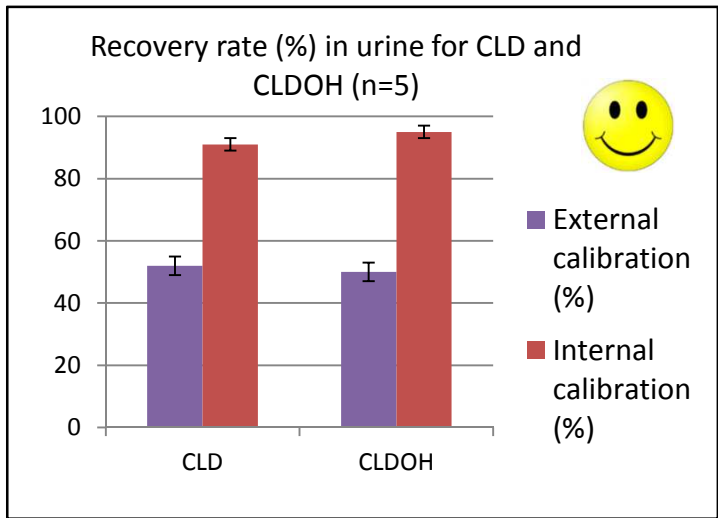
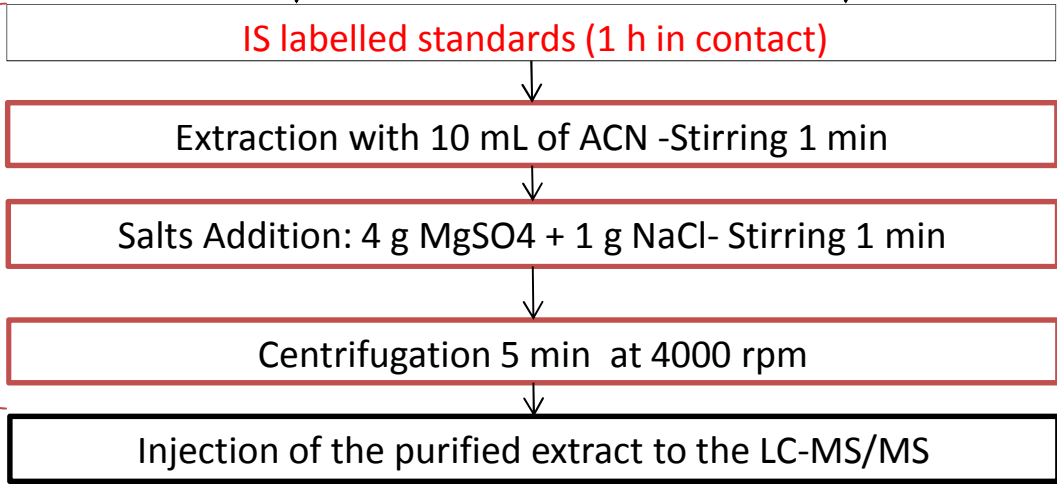


QuEChERS Transposition
for Urines and Feces
for CLD and CLDOH

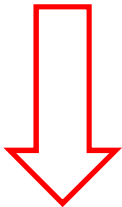
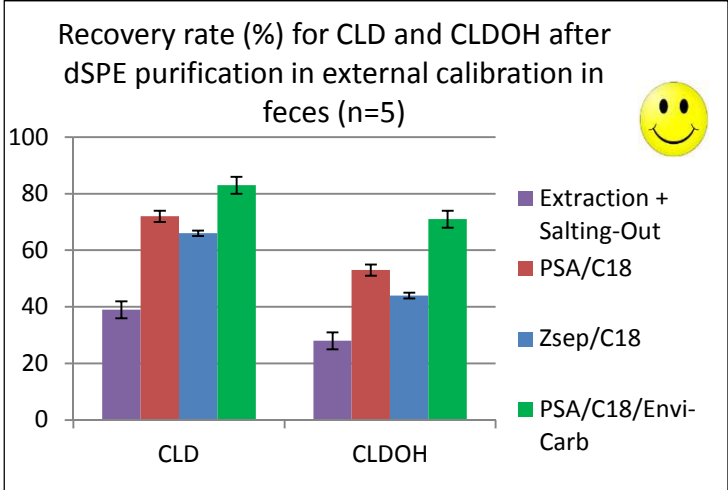
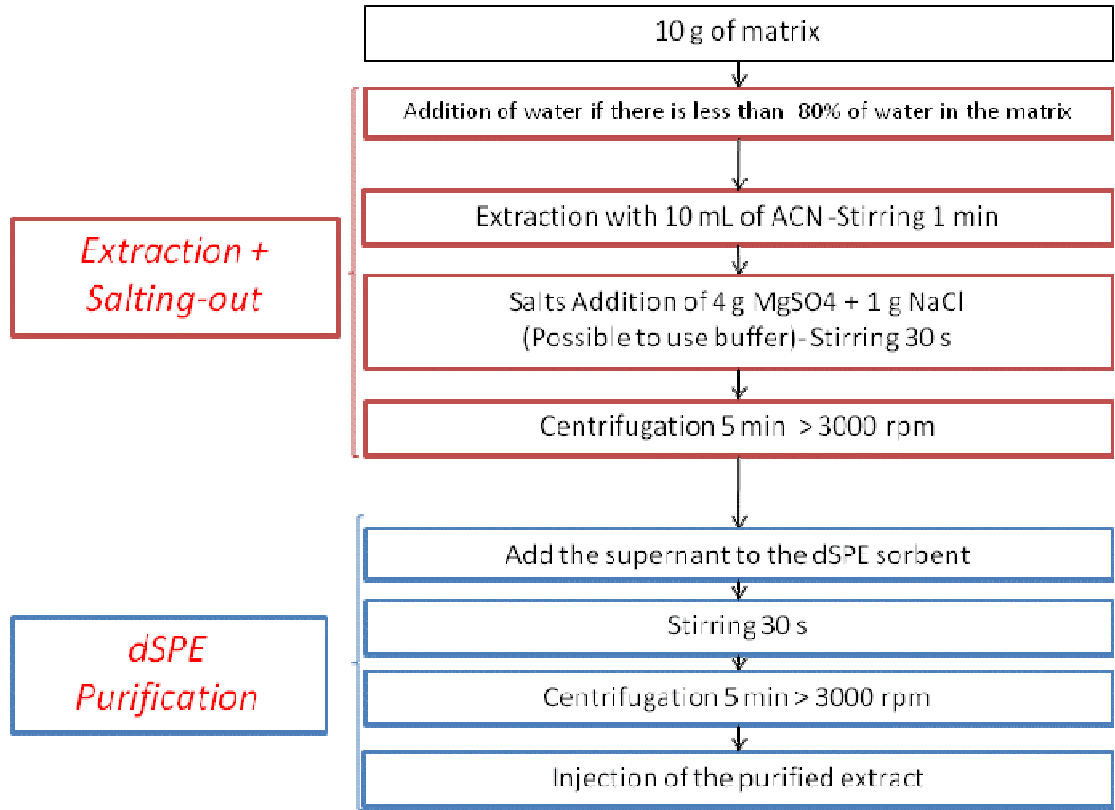
0,6 g of lyophilized feces + 4,4 mL of water + 5 mL of acetate buffer

4 mL of **urine** + 4 mL of acetate buffer

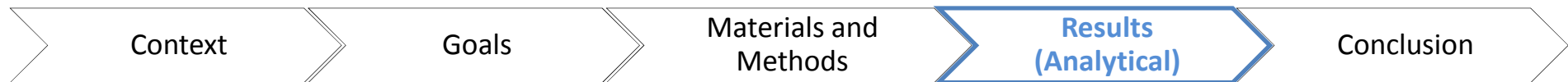
Extraction + Salting-out



QuEChERS optimisation in Feces
for **CLD** and **CLDOH**



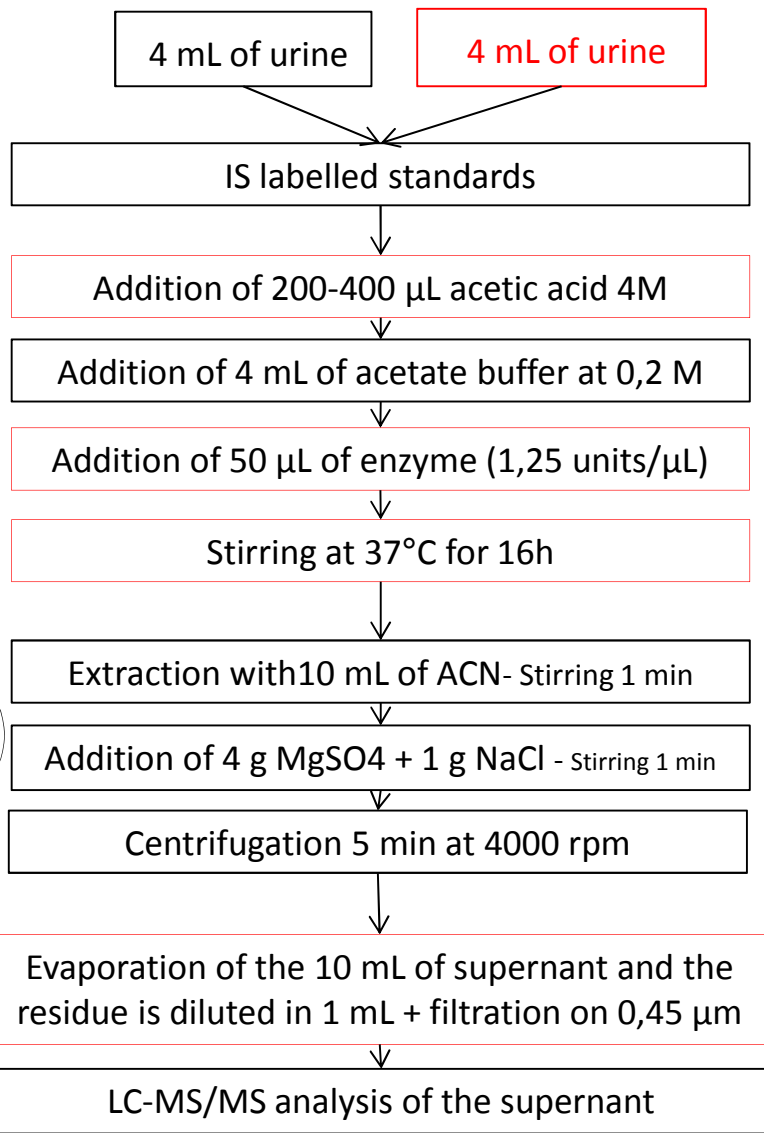
PSA/C18/Envi-Carb



**QuEChERS in Urines
for CLD, CLDOH
and conjugated metabolites**

Recoveries for CLD with evaporation:
Buffer only : 50%
Buffer + acid : 40%

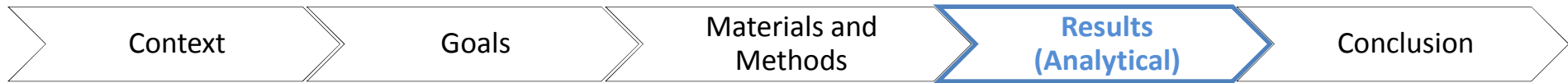
Recoveries for CLDOH with evaporation :
Buffer only : 30%
Buffer + acid : 10%



Deconjugaison

Extraction

➔ Low recoveries are obtained when acid is added for CLDOH



0,6 g of lyophilized feces + 3,4 mL of water

0,6 g of lyophilized feces + 3,4 mL of water

IS labelled standards

Addition of 50-70 µL acetic acid 4M

Addition of 4 mL of buffer acetate at 0,2 M

Addition of 50 µL of enzyme (1,25 units/µL)

Stirring at 37°C during 16h

Deconjugaison

Recoveries for CLD:
Buffer only : 80%
Buffer + acid : 70%

Addition of 2 mL of water

Extraction with 10 mL d'ACN – Stirring 1 min

Addition of 4 g de MgSO4 + 1 g de NaCl – Stirring 1 min

Centrifugation 5 min at 4000 rpm

Extraction

Recoveries for CLDOH :
Buffer only : 70%
Buffer + acid : 40%

Addition of 1 mL of the supernant to PSA/C18/Envi-Carb

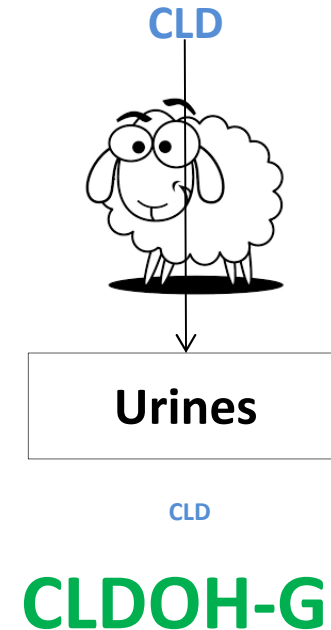
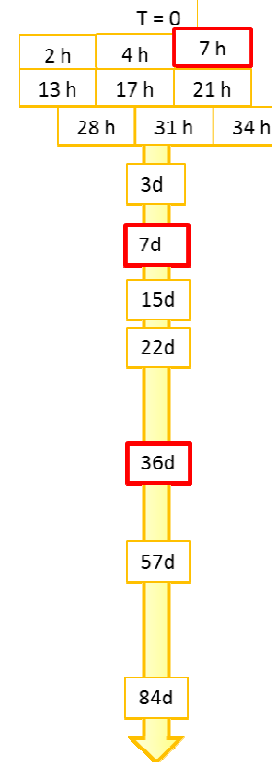
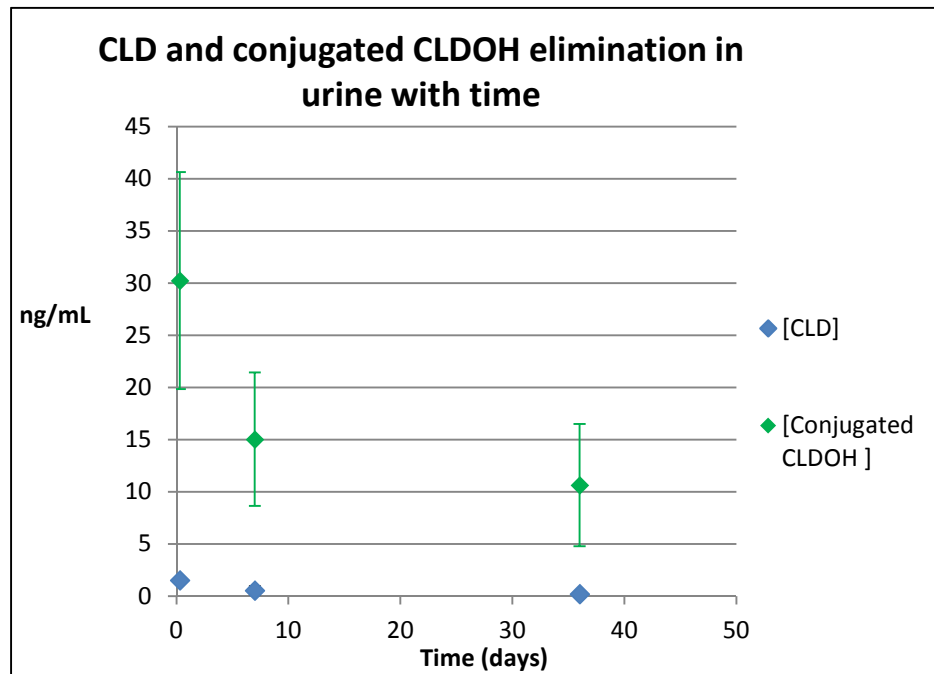
Centrifugation 5 min à 4000 rpm

Purification

LC-MS/MS analysis of the supernant

→ An evaporation step is not necessary for feces

Urinary elimination of CLD



✓ Ewe are able to metabolized CLD

✓ Global augmentation of the ratio [CLDOH-conjugated]/[CLD]

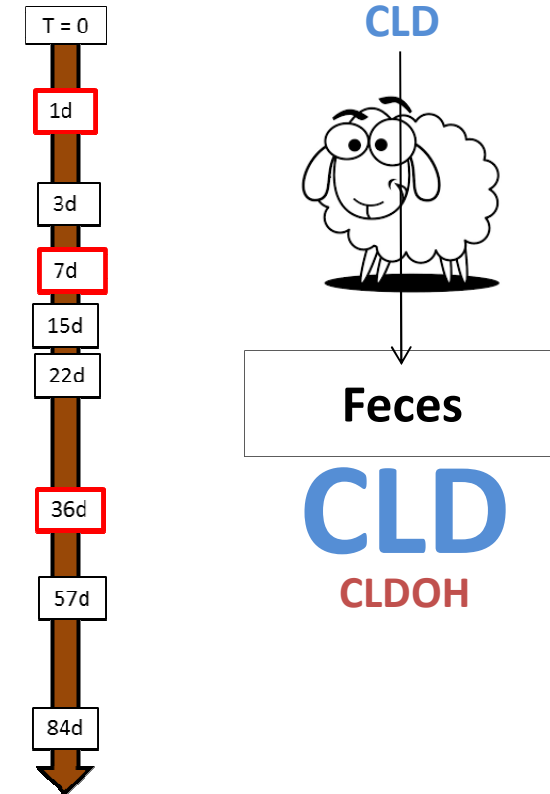
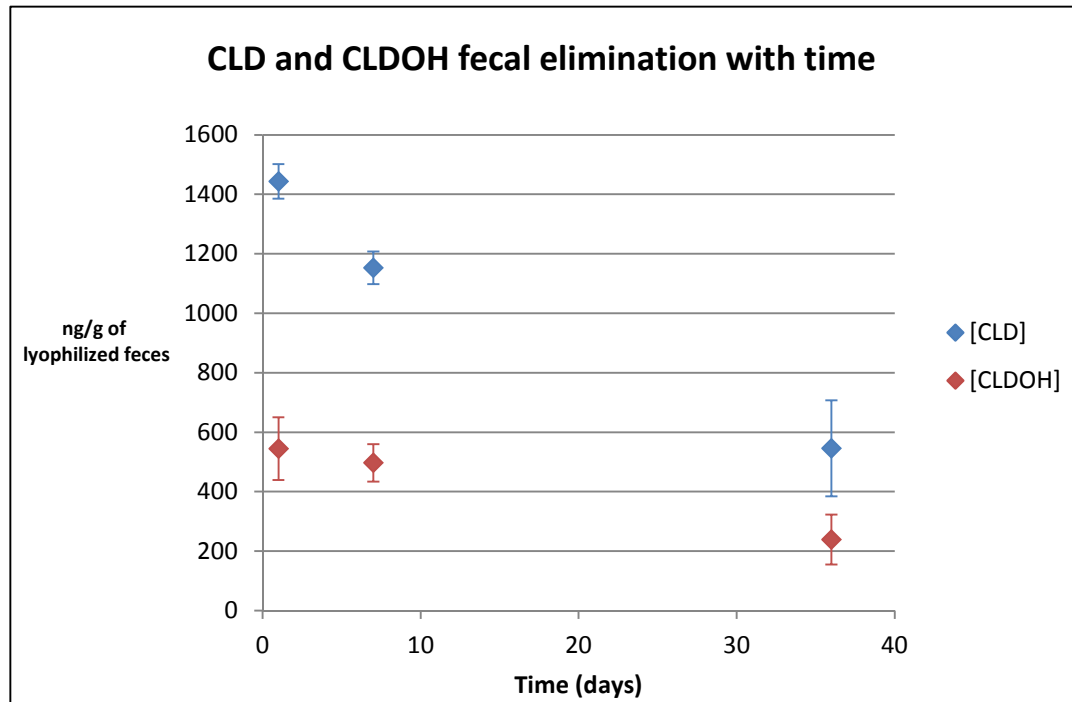
- 20/1 at 7H

- 48/1 at 7 d

- 53/1 at 36 d

Formation of CLDOH-conjugated with time

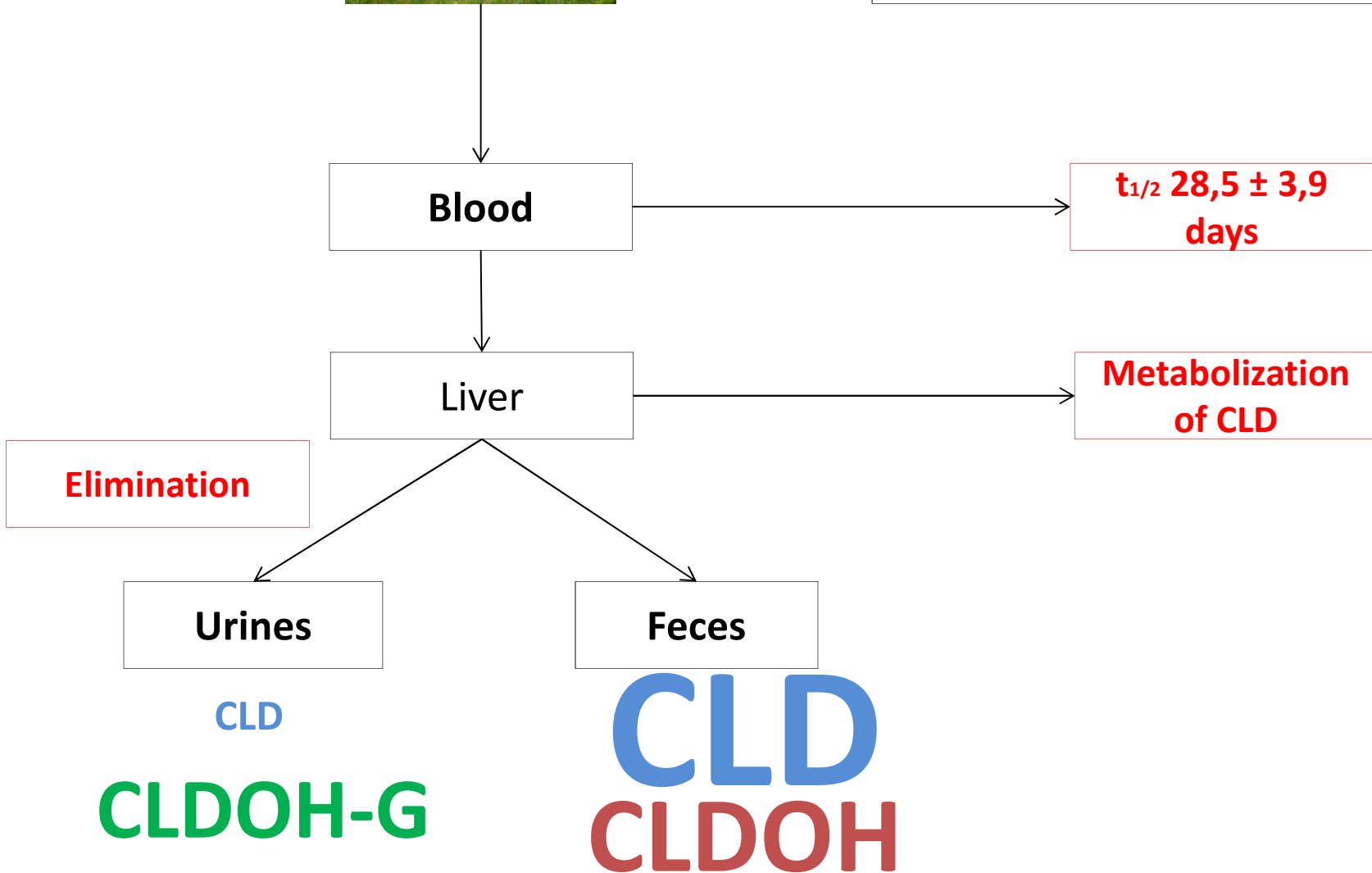
Fecal elimination of CLD



- ✓ **CLD and CLDOH are eliminated in the feces** in high concentrations as in pigs, humans and gerbils (Soine et al. 1983; Cohn et al. 1981)
- ✓ **CLD/CLDOH ratio of ~ 3/1 for ewes** close to gerbils (Houston et al. 1981) but 10/1 for pigs (Soine et al. 1981) → Difference of metabolism



✓ Development of analytical methods to analyze CLD and its metabolites in urines and feces





Thank you for your attention !

