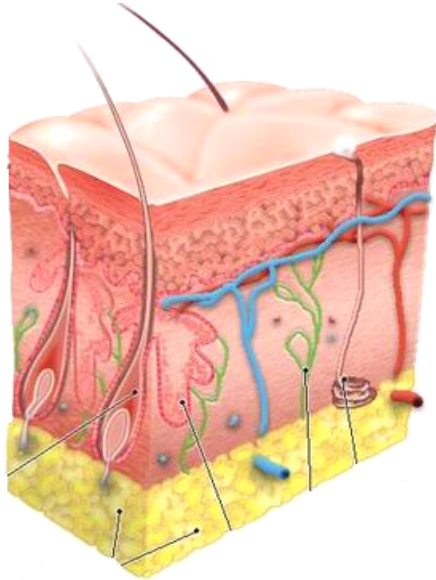


Evaluation of skin metabolism of Benzo(a)pyrene : ex vivo skin explant model validation



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D. Zalko and H. Duplan



Skin

metabolically active organ
(liver reference organ)

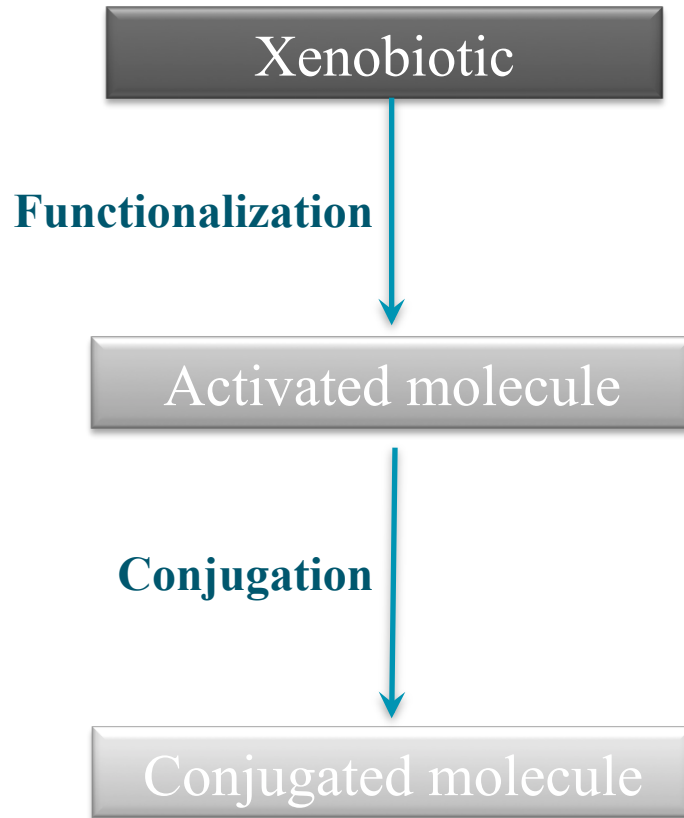


Physic and Metabolic Barrier

biotransformation of xenobiotics
Detoxify or bio-activate



I. Background and goal



Phase I enzymes

Cytochromes P450, flavines mono-oxygenases, alcohols and aldehydes deshydrogenases, esterases, epoxyde hydrolases

Phase II enzymes

UDP-glucuronyltransferases, sulfotransferases, glutathion-S-transferases, N-acétyltransferases



I. Background and goal

↳ *In the objective of complying with the European directive 76/768/CEE and with the 3R (reduce, refine, replace) concept for animal experimentation*

Pig ear skin model : Valuable alternative ex vivo model to animal experiment

Similarities with human skin (“gold standard”)
(Histological and biochemical properties)

Recognized for its use in skin penetration studies



Characterization of metabolic activity of ex vivo pig ear skin model coupled with barrier effect

Detailed study of the distribution and metabolism of model xenobiotics



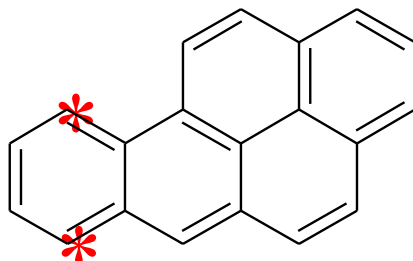
Choice of model xenobiotics

- different physicochemical properties
- well-known metabolism
- involving Phase I and II enzymes

Benzo(a)pyrene :

- polycyclic aromatic hydrocarbon
- absorbed following inhalation, oral, and dermal routes
- highly metabolized in the liver into several metabolites
- in particular, the 7,8-diol-9,10-epoxyde could be responsible of the carcinogenic effect of B(a)P

[B(a)P]



Log K_{ow} 6.35



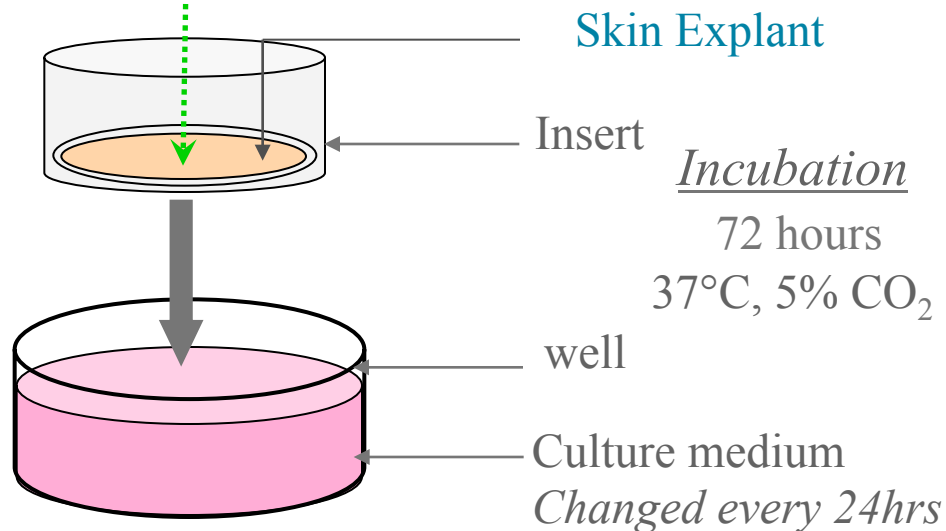
II. Experimental approach

Schema of skin short-term culture

domestic pig ear
skin
(Slaughterhouse)

radioactive molecules

50, 100, 200, 400, 600 and 800 nmol



Section

500 μ m thick



6-well plates

Inserts 4.1 cm²

1.5ml culture medium

Sampling

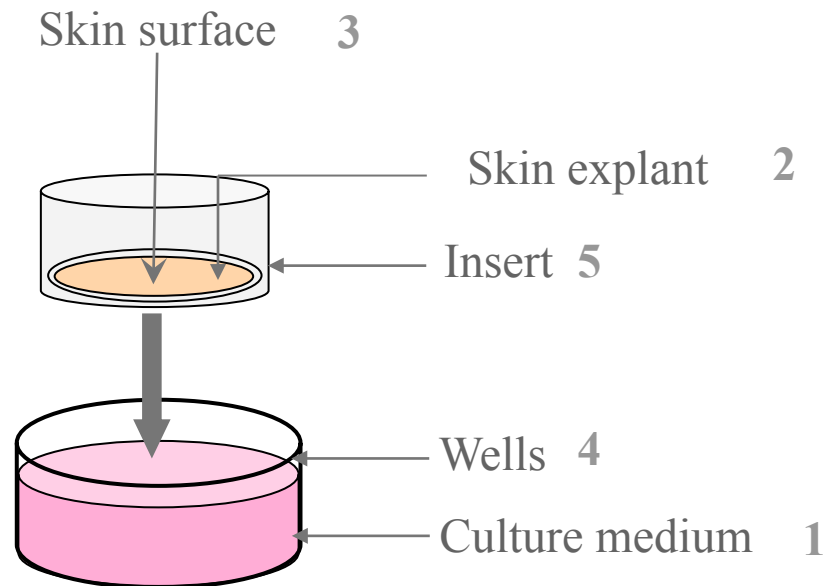
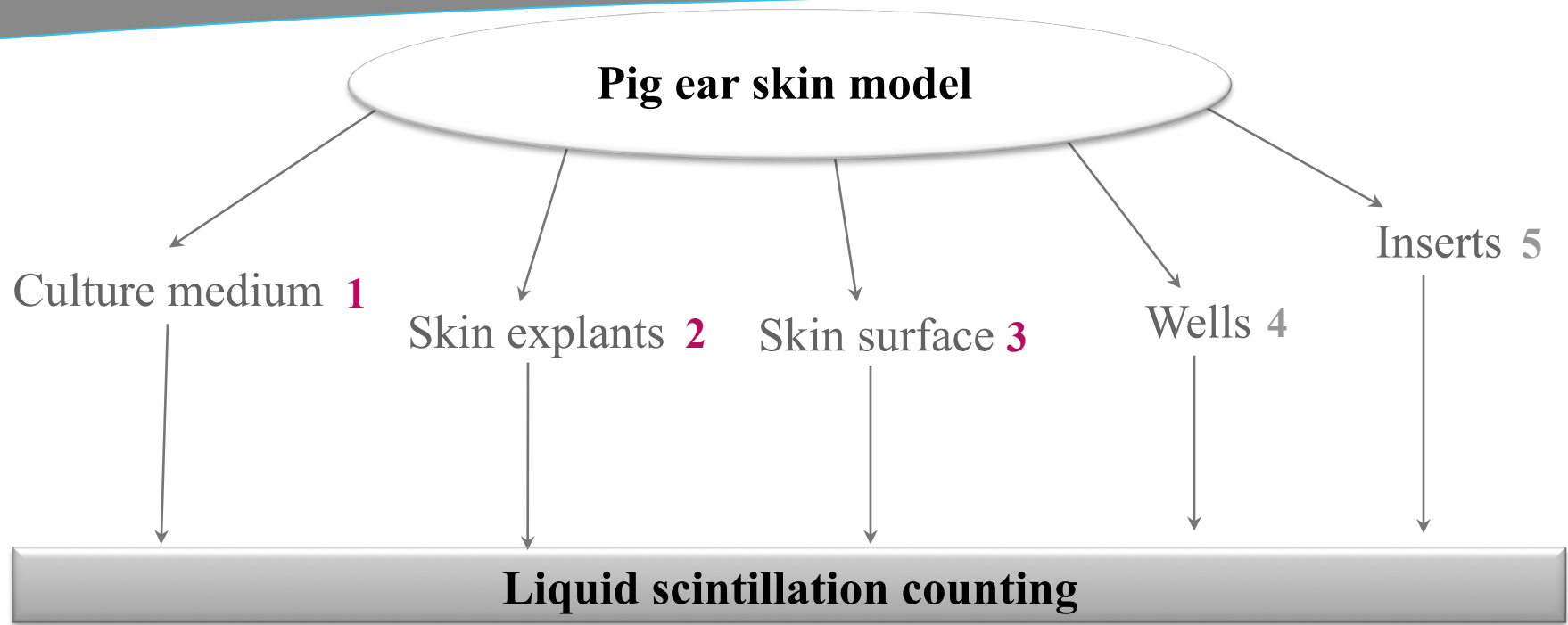
Culture medium : 0, 24, 48, 72 hr

All compartments : 72hr

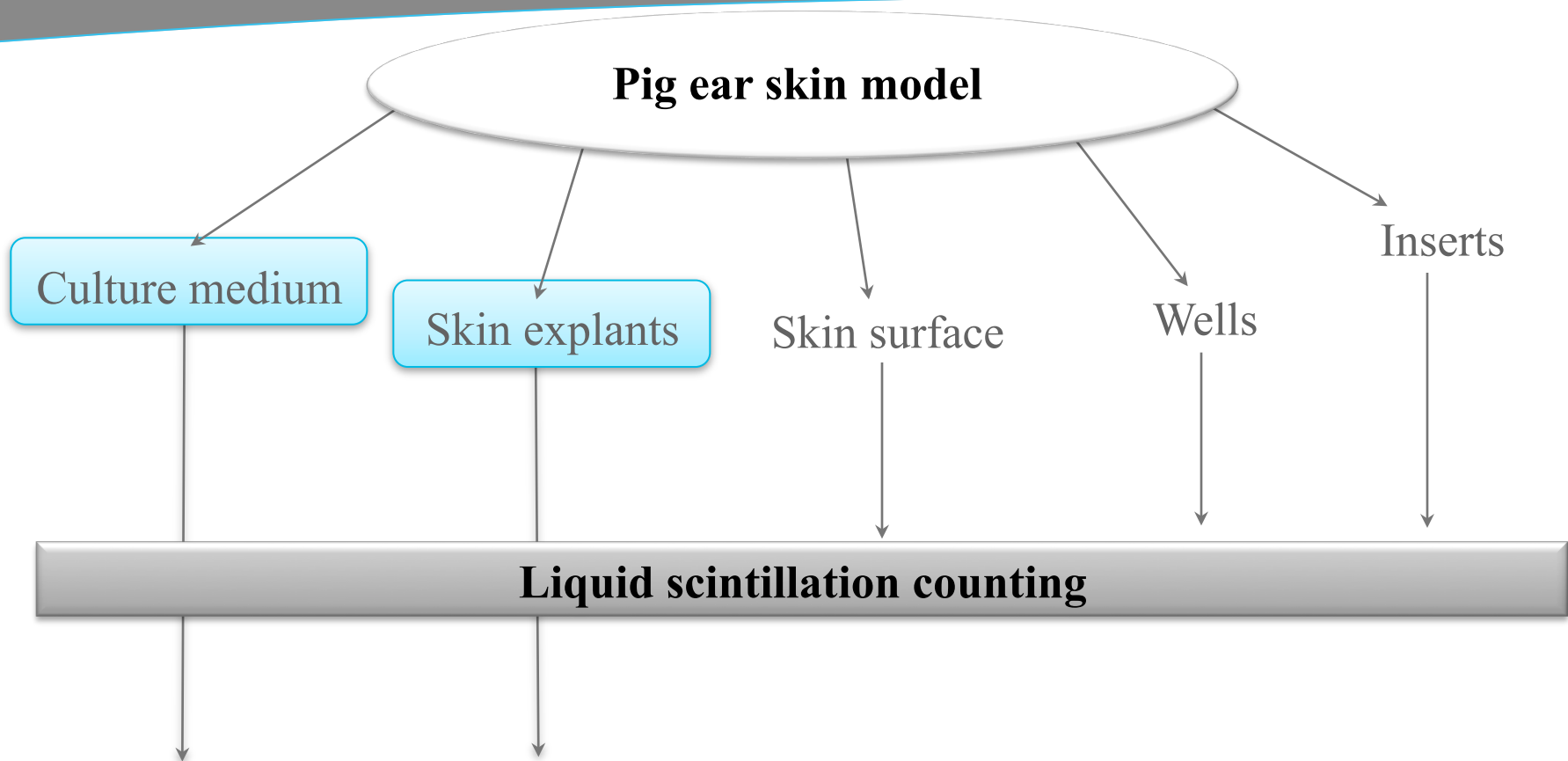


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II. Experimental approach



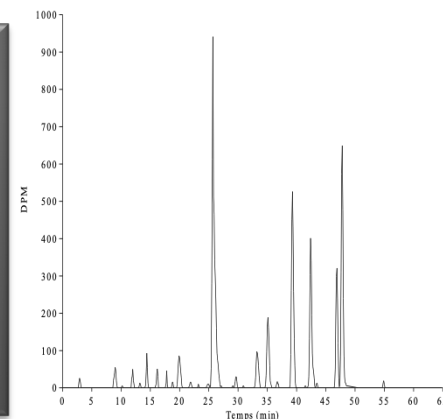
II. Experimental approach



Metabolites detection and quantification : Radio-HPLC

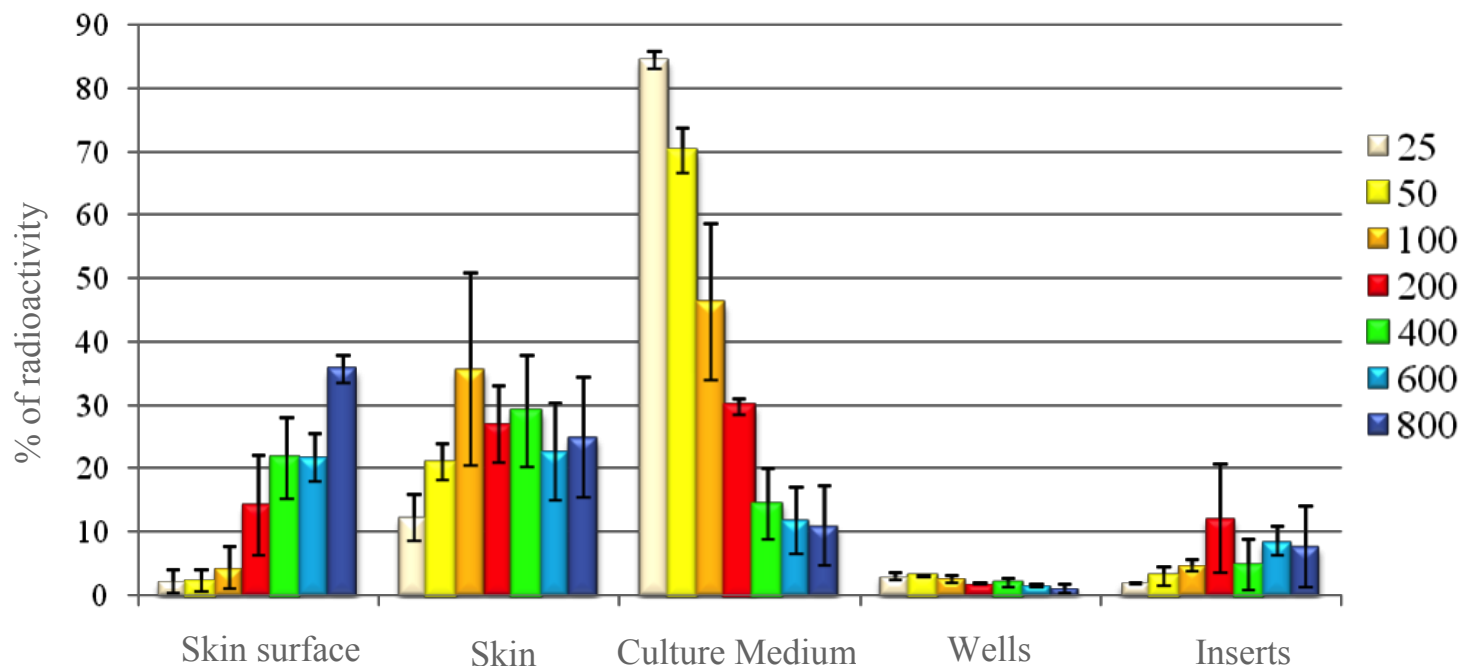
Metabolites identification : Mass spectrometry, NMR

Enzymatic hydrolysis



Compartmental radioactivity levels

Time : 24, 48 et 72 h



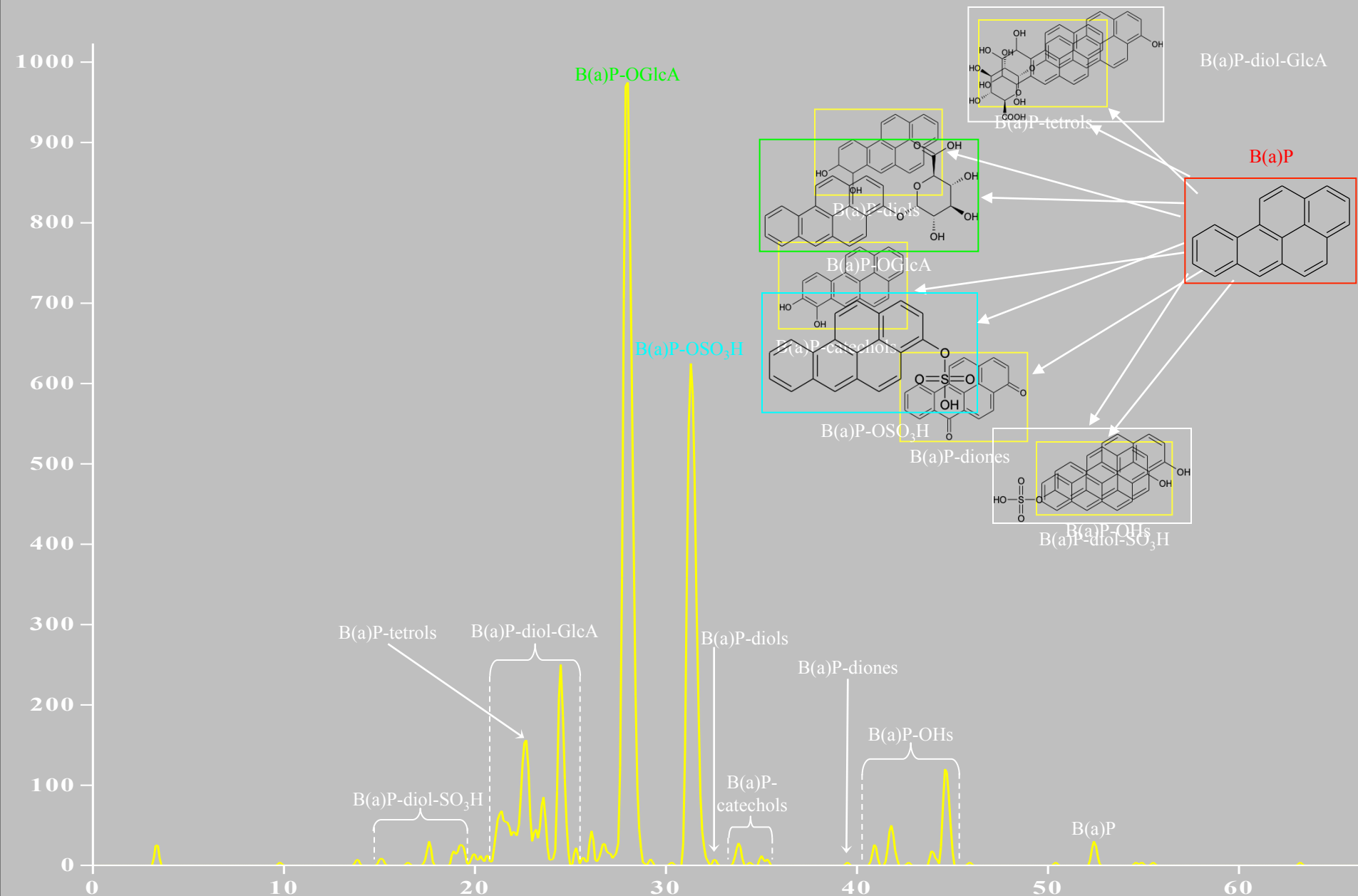
- Radioactivity:

Low doses (25 to 100 nmoles): culture media ($46 \pm 5\%$ to $85 \pm 1\%$)

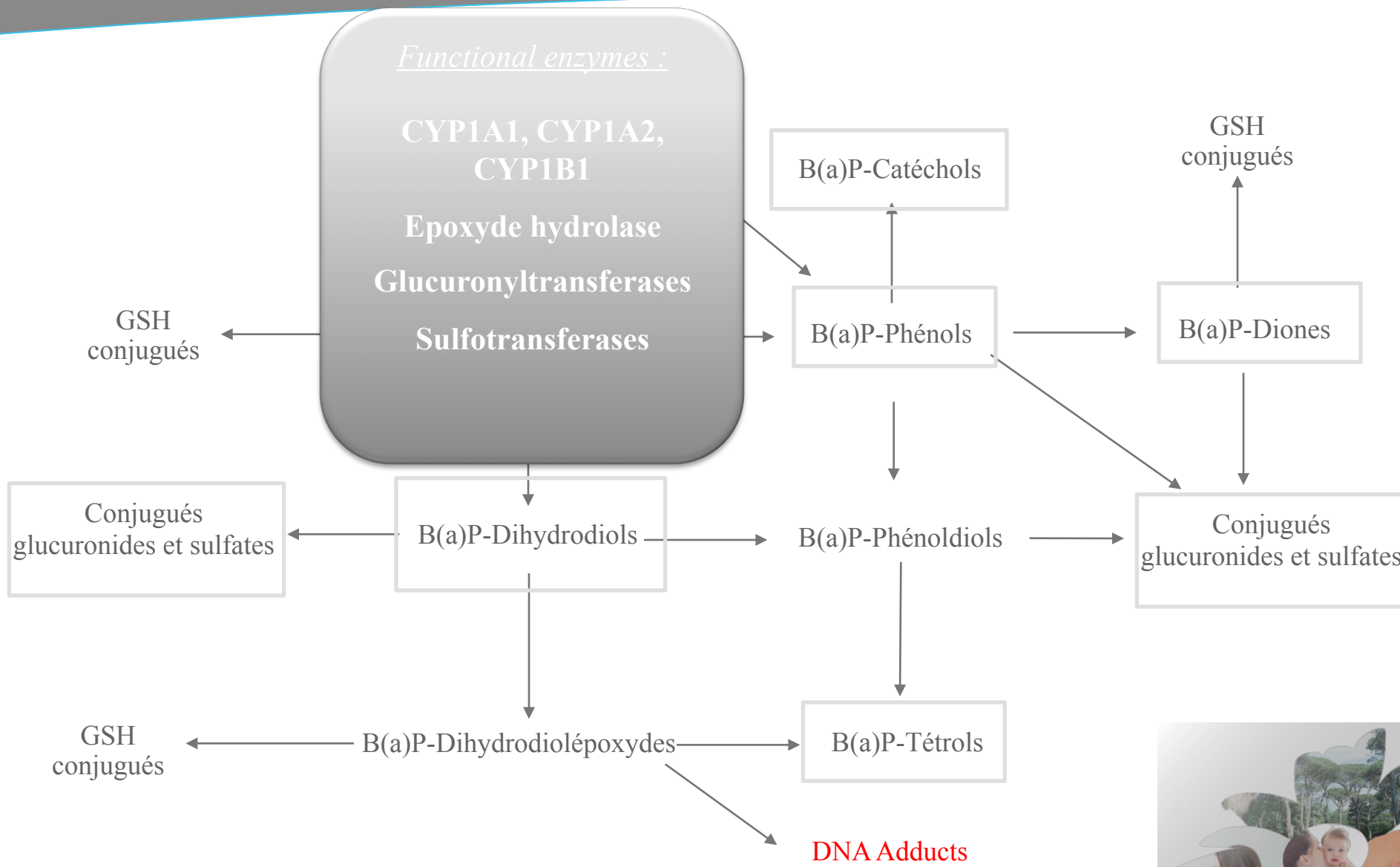
High doses (200 to 800 nmoles): surface + skin ($41 \pm 8\%$ to $61 \pm 2\%$)



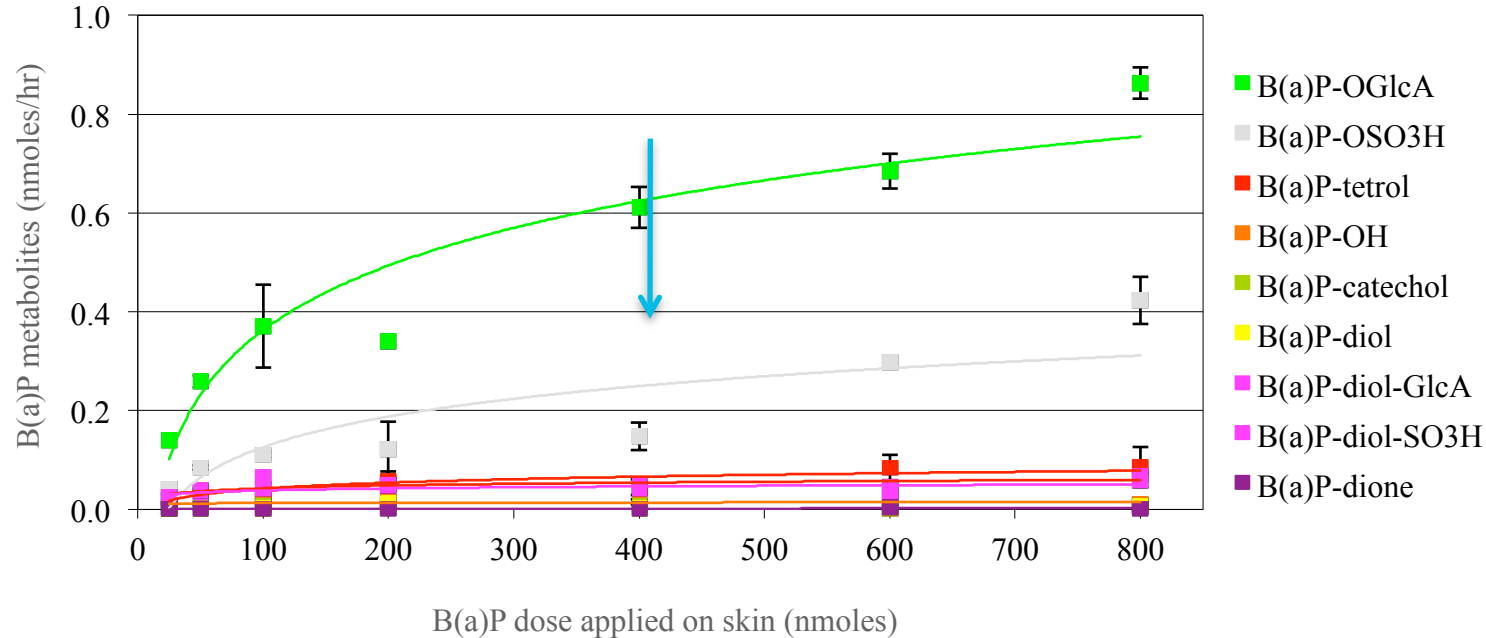
III. Results : Qualitative analysis



III. Results : BaP metabolic pathway in our skin model



III. Results : kinetic of metabolite formation



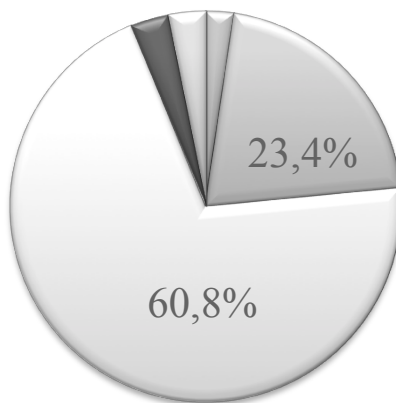
- **Major metabolites**: glucuronide and sulfates conjugates
- Michaelis Menten curves
- **Enzymatic saturation**: 200nmoles of B(a)P



IV. Influence of BaP metabolism on its absorption

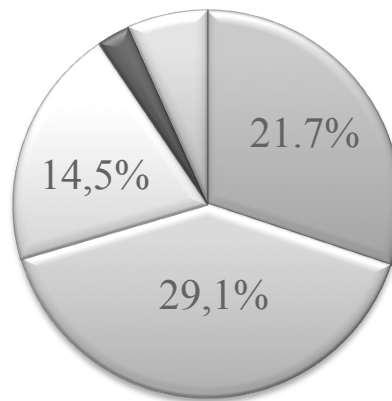
50 nmoles

B(a)P
Log K_{ow} 6,5

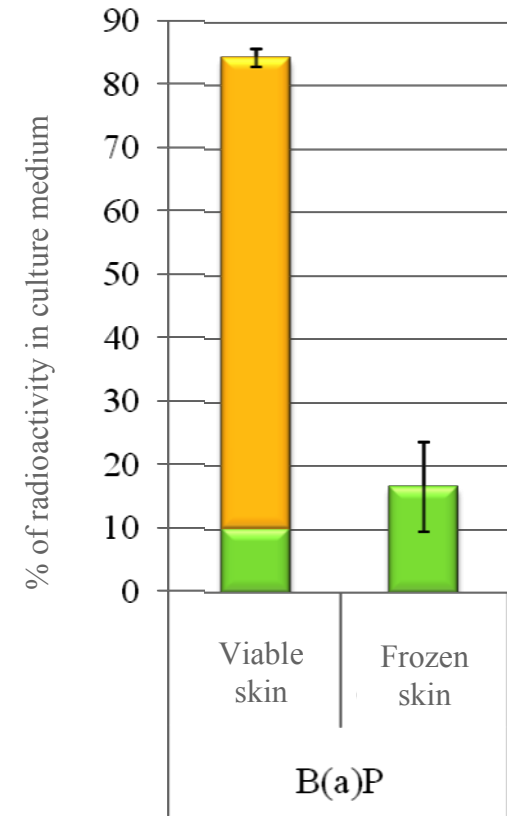
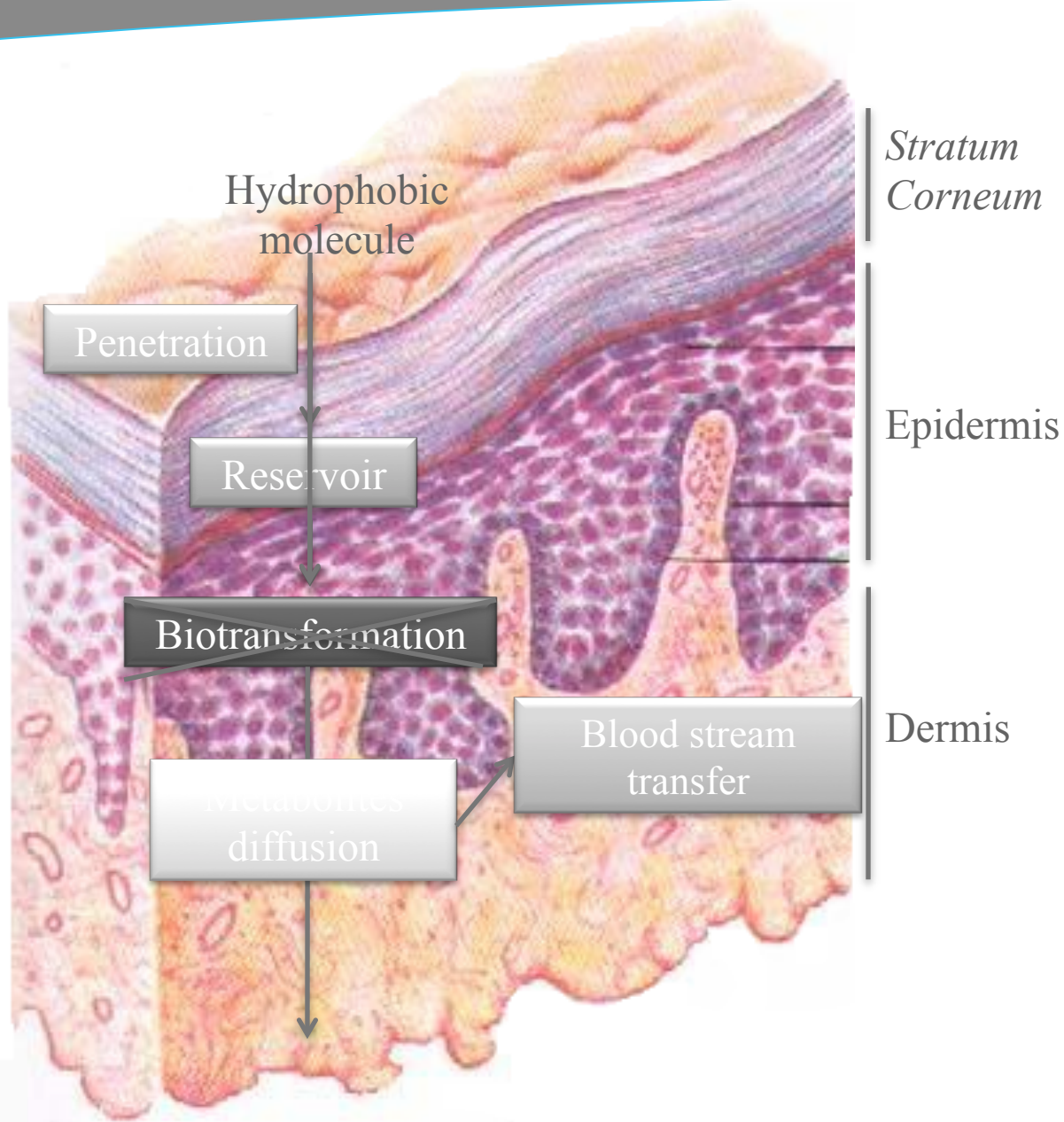


- Skin surface
- Skin
- Culture medium
- Insert
- Wells

400 nmoles



IV. Influence of BaP metabolism on its absorption



V. Conclusions

- The percutaneous diffusion rate of a compound depends not only on its physico-chemical properties, but also on the expression of functional enzymes in the skin, especially for hydrophobic compounds like benzo(a)pyrene
- Biotransformation at the level of the skin could be an important factor for determining the overall diffusion rate of a xenobiotic
- As in the liver, benzo(a)pyrene was metabolized in the skin in several products as diols, tetrols that could induce skin sensitization or skin cancers.
- We performed further studies with several known molecules that validated the ex vivo pig ear skin model (testosterone, 7-EC, Caffeine...)

Jacques et al; Toxicol In Vitro 2010; SPP 2013 accepted



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