



**HAL**  
open science

## Metabolic balance of [3H]-BADGE in rodents: A spotlight on the complex in vivo fate of the diglycidyl ether analogue of bisphenol A

Elodie Person, Isabelle Jouanin, Emilien Jamin, Florence Blas-Y-Estrada, Sandrine Bruel, Jean-Pierre Cravedi, Nicolas Cabaton, Daniel Zalko

### ► To cite this version:

Elodie Person, Isabelle Jouanin, Emilien Jamin, Florence Blas-Y-Estrada, Sandrine Bruel, et al.. Metabolic balance of [3H]-BADGE in rodents: A spotlight on the complex in vivo fate of the diglycidyl ether analogue of bisphenol A. IUTOX 15th International Congress of Toxicology, Jul 2019, Honolulu (HI), United States. hal-04672542

**HAL Id: hal-04672542**

**<https://hal.science/hal-04672542v1>**

Submitted on 19 Aug 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

Elodie N. Person<sup>1</sup>, Isabelle Jouanin<sup>2</sup>, Emilien L. Jamin<sup>2</sup>, Florence Blas-Y-Estrada<sup>1</sup>, Sandrine Bruel<sup>1</sup>, Jean-Pierre Cravedi<sup>1</sup>, Nicolas J. Cabaton<sup>1</sup> and Daniel Zalko<sup>1</sup>

INRA Toxalim (Research Centre in Food Toxicology), Université de Toulouse, INRA, ENVT, INP-Purpan, UPS, 180 chemin de Tournefeuille, 31027 Toulouse, France

<sup>1</sup>Metabolism and Xenobiotics (MeX) Team, <sup>2</sup>Axiom-Metatoul Team

## Introduction

### Bisphenol A diglycidyl ether = BADGE

(2,2-Bis[4-(glycidyloxy)phenyl]propane, CAS N°1675-54-3, MW 340.4)

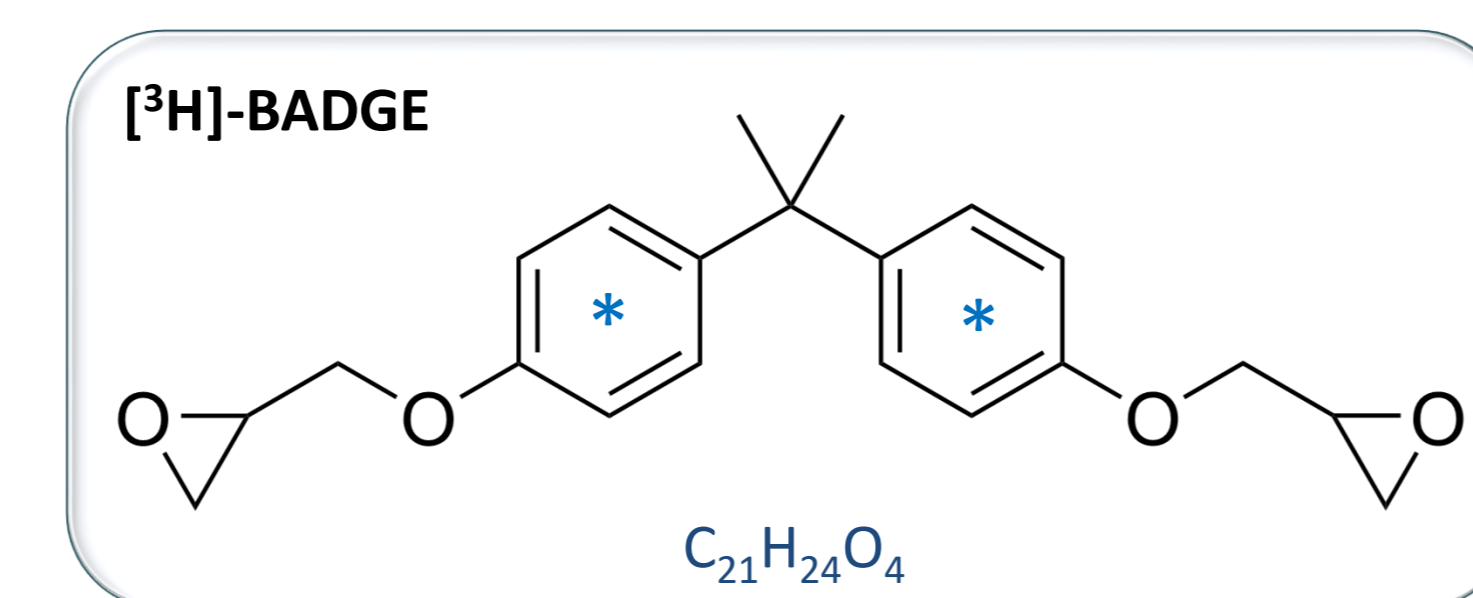
- Used in the manufacture of **epoxy resins** and **polycarbonates** for **food packaging**
- Synthesized by a condensation reaction between **epichlorohydrin** and **Bisphenol A**
- Protective coatings**: car and marine industry, food packaging, adhesives, dental sealants, etc.<sup>[1, 2]</sup>
- Reported **human daily intake**: approximately **0.16 µg/kg b.w./day**<sup>[2, 3]</sup>
- Tolerable Daily Intake, BADGE + hydrolysis products = 0.15 mg/kg b.w.<sup>[4]</sup>
- Migration** of BADGE + hydrolysis products does not exceed: 9 mg/kg in food, 1 mg/kg for chlorinated derivatives<sup>[4]</sup>
- Possible **endocrine disruptor** (PPARγ antagonist)<sup>[5, 6]</sup>



**Objectives:** Investigate the **fate of BADGE** in male and female Wistar rats  
Explore **BADGE metabolic balance** and **identify the different metabolic pathways** involved in its **biotransformation** for **toxicological purposes**  
**Identify candidate biomarker metabolites**, with the perspective of further human biomonitoring studies

## Methodology

### Chemicals:



[<sup>3</sup>H]-BADGE (radiochemical purity > 97 %, specific activity 10.9 GBq/mmol) was synthesized in-house from radio-labelled [<sup>3</sup>H]-Bisphenol A (Moravek). Unlabeled BADGE was purchased from Sigma Aldrich.

### Animal experiment:

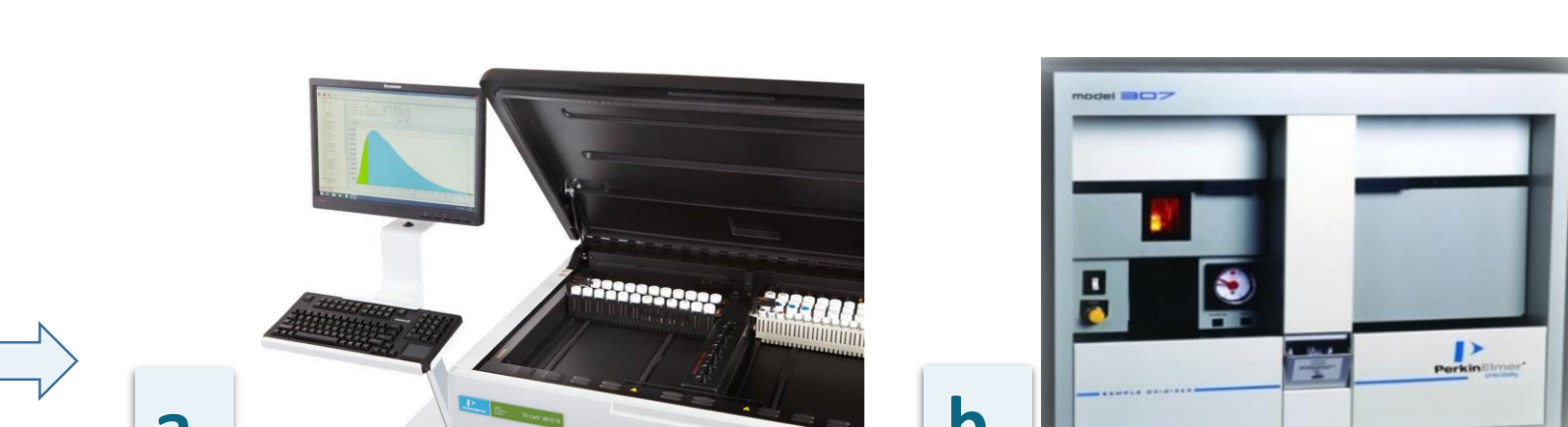
 50 µg/kg [<sup>3</sup>H]-BADGE (gavage)


**3 male and 3 female** 8-week old Wistar rats were force-fed an adjusted single [<sup>3</sup>H]-BADGE dose of **50 µg/kg b.w.** (1.6 MBq/kg b.w.) dissolved in corn oil. After **24h** spent in individual stainless steel metabolic cages, with water and food supplied *ad libitum*, rats were weighed and euthanized.

### Metabolic Balance and Tissue Distribution:



Urine, feces, blood, liver, genital tract, glands, adipose tissue, muscle, digestive tract (and its content), brain, kidney, spleen, lung, heart, bladder, pancreas, skin and the remaining carcass were **collected for radioactivity quantification**. **Extractions** were performed on feces and digestive contents. Radioactivity was measured in liquid samples using a **Packard scintillation analyzer** (a.)



Tri-Carb 2910TR, with Packard Ultima Gold as the scintillation cocktail (Perkin Elmer). Feces were dried and ground before **solubilization** in **Soluene** (Perkin Elmer). Radioactivity in homogenized tissues, organs, blood, and carcasses was determined by complete **combustion** using an **Oxidizer 307** (Perkin Elmer, b.), followed by quantification on a scintillation analyzer. At least 3 replicates were analyzed for each sample.

### Analytical conditions:

Flow rate	1 mL/min	Column temperature	25 °C	Analysis time	55 min
<b>Phases</b>	A: Ammonium acetate buffer 20 mM pH 3,5 / Acetonitrile (95:5 v/v) B: Ammonium acetate buffer 20 mM pH 3,5 / Acetonitrile (10:90 v/v)				
<b>Gradient</b>	0-4 min: 100 % A, 4-6 min: 100-75 % A, 6-16 min: 75 % A, 16-18 min: 75-70 % A, 18-25 min: 70 % A, 25-27 min: 70-30 % A, 27-38 min: 30 % A, 38-40 min: 30-0 % A, 40-50 min: 0 % A, 50-51 min: 0-100 % A, 51-55 min: 100 % A				

### Metabolic profiling and identification:

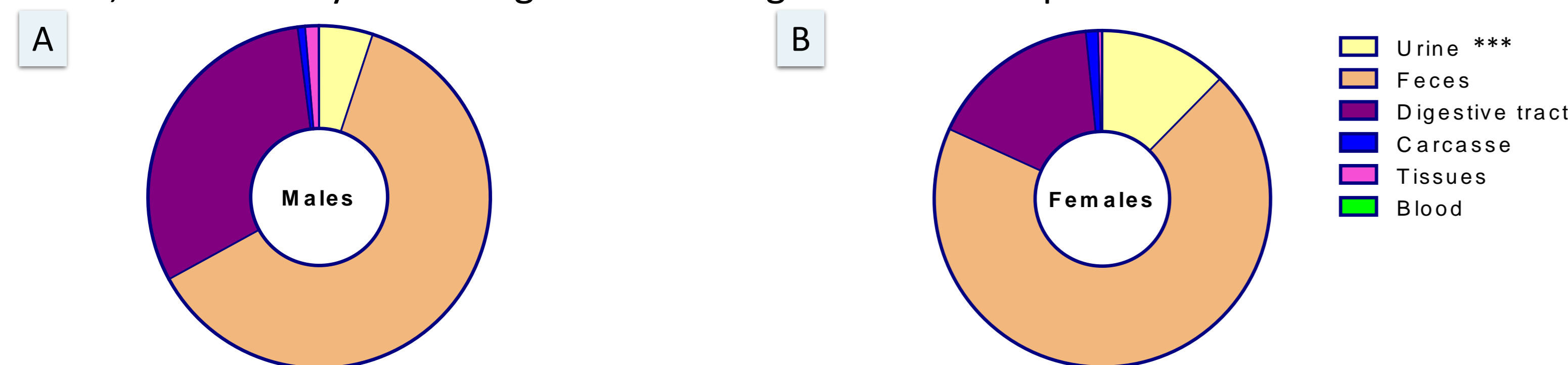
**High-performance-liquid-chromatography coupled to on-line radioactivity detection (Radio-HPLC)**. Excreted metabolites were separated on an Ultimate 3000 (ThermoScientific, c.) HPLC system, with a Zorbax SB-C18 column (5 µm ; 4.6 x 250 mm, Agilent) coupled to a Flo-One A610TR detector (Perkin Elmer, d.), using 2 mL/min Flo-Scint II (Perkin Elmer) as the scintillation cocktail.



## Preliminary results

### Metabolic balance of [<sup>3</sup>H]-BADGE following a single oral dose administered to male and female Wistar rats

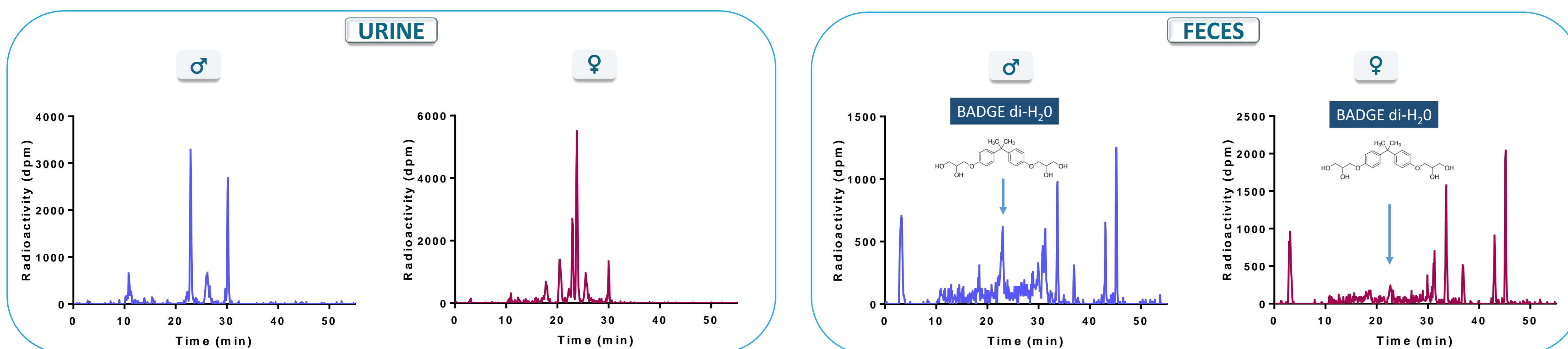
For each animal, radioactivity remaining in the feeding material was quantified to calculate the nominal [<sup>3</sup>H]-BADGE dose administered: **46.8 ± 2.2 µg/kg b.w.** (1.5 ± 0.07 MBq/kg b.w.)



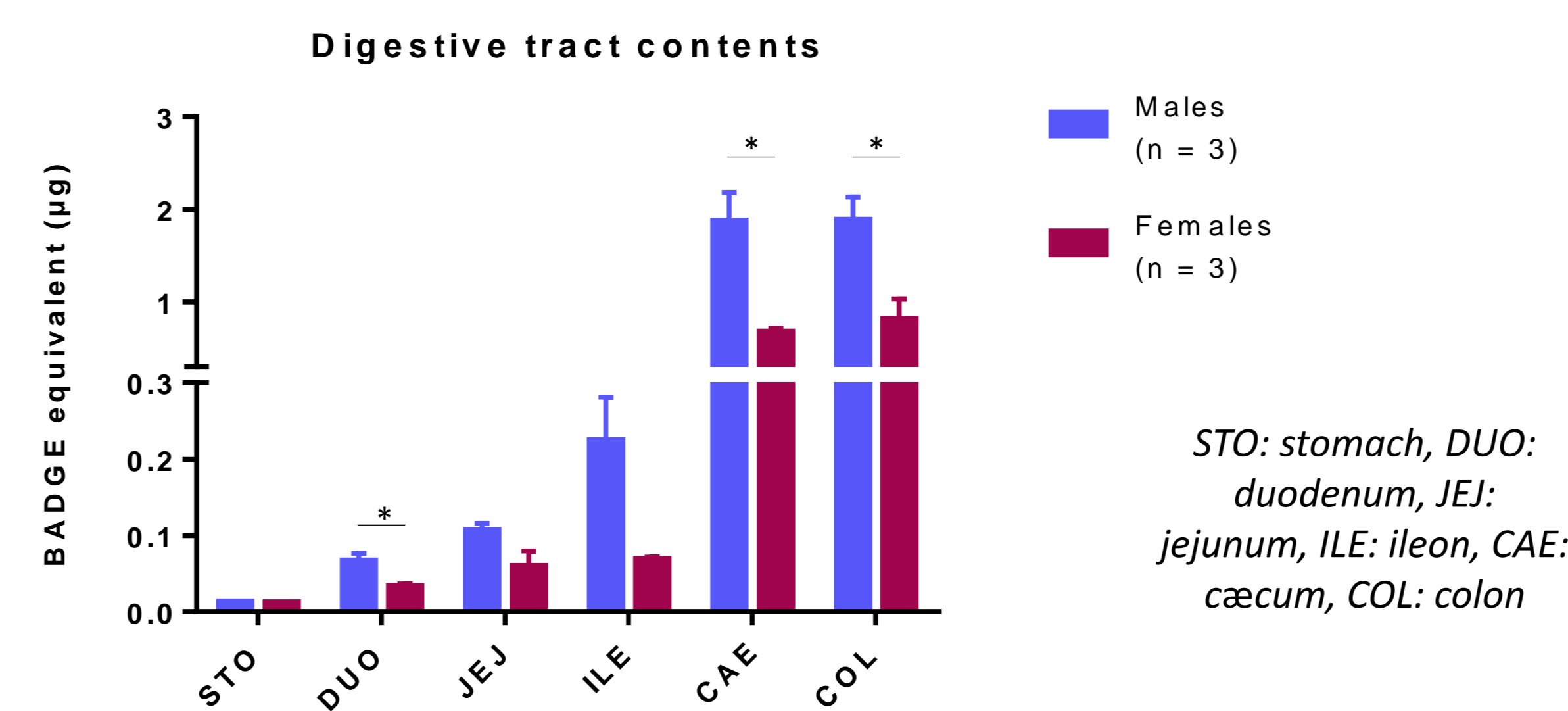
**Fig. 1: 24h metabolic balance** in male (A) and female (B) Wistar rats. Results (mean, n = 3 for each group) are expressed as % of the administered radioactivity (*t*-test, \*\*\* *p*-value ≤ 0.001, Graphpad®)

- 92.1 ± 9.4 %** of the administered radioactivity recovered at 24h following BADGE administration (n = 6).
- Feces was the main route of excretion** (55.1 ± 16.8 % in males, 66.2 ± 6.7 % in females), and the **digestive tract** accounted for 27.5 ± 6.7 % of the administered radioactivity in males and 15.8 ± 4.1 % in females, respectively.
- Significant sex-related differences were observed for radioactivity recovery in **urine**: 4.5 ± 0.9 % in males and 11.8 ± 0.9 % in females, respectively.

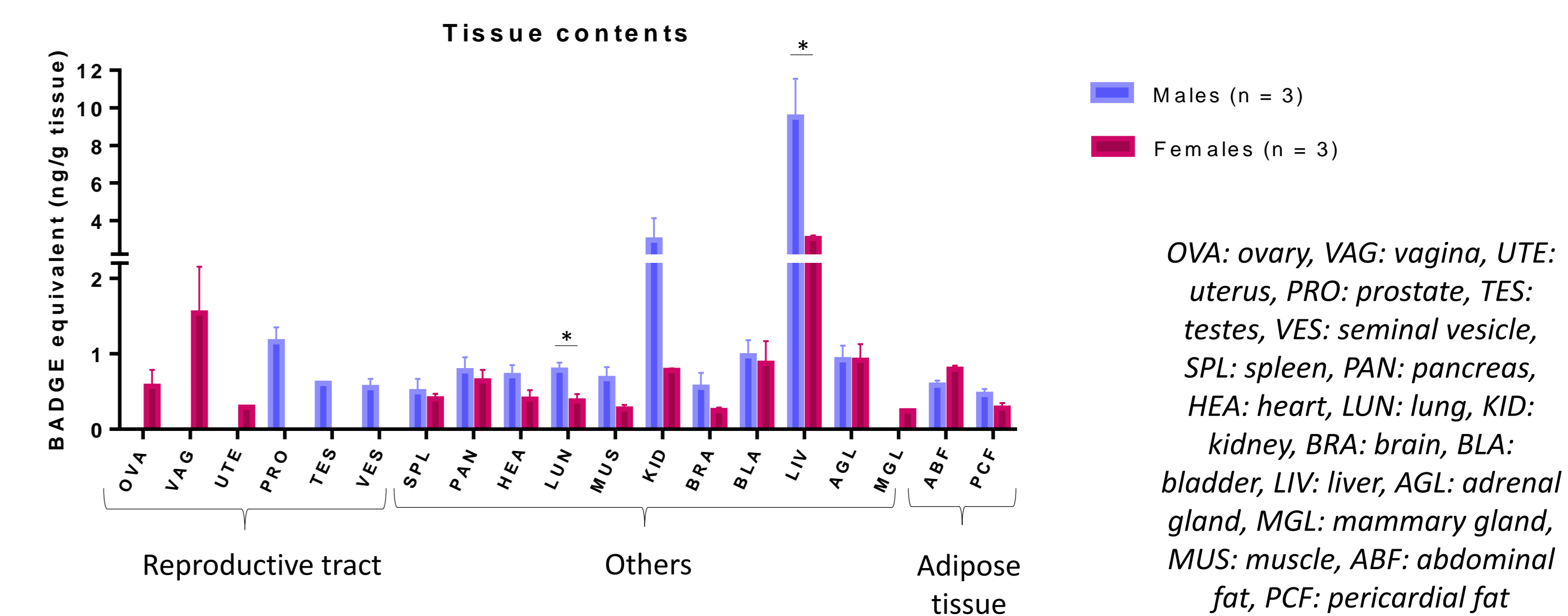
### Radiochromatographic profiling of urine and feces



**Fig. 4: Typical Radio-HPLC profiles** for urine and feces collected over 24h



**Fig. 2: 24h metabolic balance:** residual radioactivity in digestive tract contents of male and female Wistar rats. Results (mean ± SEM) are expressed in µg of BADGE equivalent (*t*-test, \* *p*-value ≤ 0.05, Graphpad®)



**Fig. 3: Residual levels of radioactivity** in tissues of male and female Wistar rats. Results (mean ± SEM) are expressed in ng of BADGE equivalent/g tissue (ppb) (*t*-test, \* *p*-value ≤ 0.05, Graphpad®)

- Sex-related differences** were highlighted for **urine** and **fecal metabolite profiles**
- Complex radio-chromatographic profiles underline the **chemical reactivity** of BADGE

## Conclusions

In this **first *in vivo* 24h-study** of [<sup>3</sup>H]-BADGE carried out in male and female Wistar rats, radioactivity was found to be **excreted** mainly in **feces**, with residues found in all tissues, including reproductive organs.

**Sex-related differences** were highlighted for **urine, liver, lung** as well as **duodenum, caecum** and **colon contents**. Qualitative differences are also suggested by first Radio-HPLC analyzes carried out for urine and feces.

BADGE elimination over 24h (urine + feces) appears to be faster in female rats compared to males (77.9 ± 6.2 % in females vs. 59.6 ± 16.2 % in males).

Preliminary HPLC Radio-profiling analyzes strongly suggest a **complex metabolism** of this molecule. **Further HPLC-MS/MS investigations** are being carried out, with the aim to identify unknown **BADGE metabolites**.

## References

- Xue J. et al., 2018, Environ Res. 162:35-40.
- Poole A. et al., 2004, Food Addit Contam. 21(9):905-19.
- Wang L. et al., 2015, Environ Sci Technol. 49(5):3150-7.

- Commission regulation N°1895/2005 on the restriction of use of certain epoxy derivatives in materials and articles intended to come into contact with food, 2005.
- Wright HM. et al., 2000, J Biol Chem. 275(3):1873-7.
- Satoh K. et al., 2004, Food Chem Toxicol. 42(6):983-93.

All the experimental procedure was carried out in accordance with the accepted standards of animal care and validated by the local ethical committee for animal experimentation N°086 (project number: APAFIS#1308-201706161630824 v3) and by the French Ministry of Higher Education, Research and Innovation (agreement: C3155513)