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

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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.^[1, 2]
- Reported **human daily intake**: approximately **0.16 µg/kg b.w./day**^[2, 3]

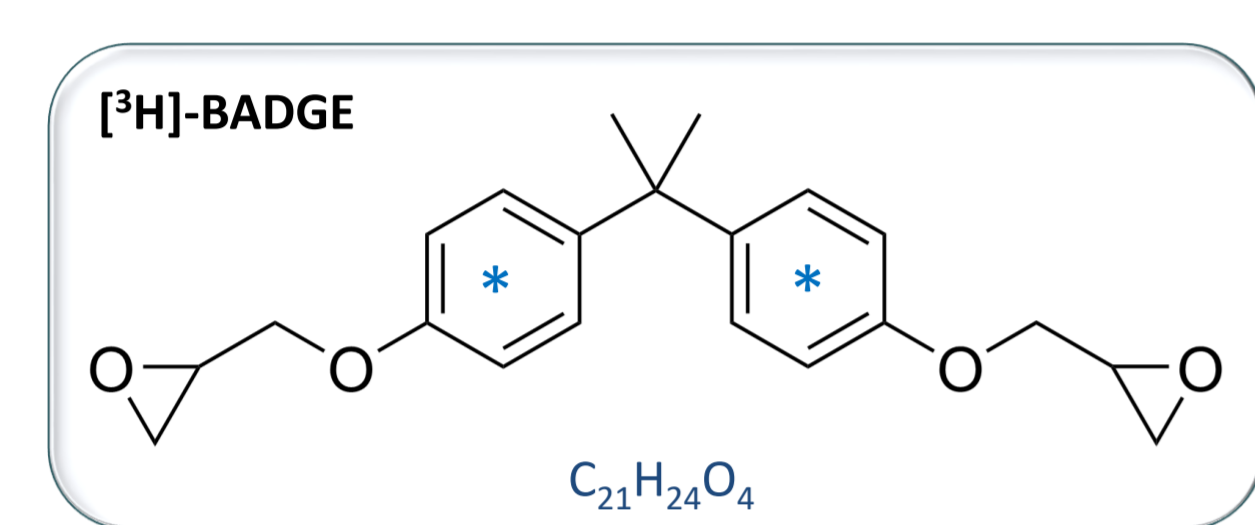


- Tolerable Daily Intake, BADGE + hydrolysis products = 0.15 mg/kg b.w.^[4]
- Migration** of BADGE + hydrolysis products does not exceed: 9 mg/kg in food, 1 mg/kg for chlorinated derivatives^[4]
- Possible **endocrine disruptor** (PPARγ antagonist)^[5, 6]

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:



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

Animal experiment:

50 µg/kg [³H]-BADGE (gavage)

3 male and 3 female 8-week Wistar rats were forced an adjusted single [³H]-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 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** (b, Perkin Elmer), followed by quantification on a scintillation analyzer. At least 3 replicates were analyzed for each sample.

Metabolic profiling and identification:

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

Analytical conditions:

Flow rate	1 mL/min	Column temperature	25 °C	Analysis time	55 min
Phases	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)				
Gradient	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				

Preliminary results

Metabolic balance of [³H]-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 [³H]-BADGE dose administered: **46.8 ± 2.2 µg/kg b.w.** (1.5 ± 0.07 MBq/kg b.w.).

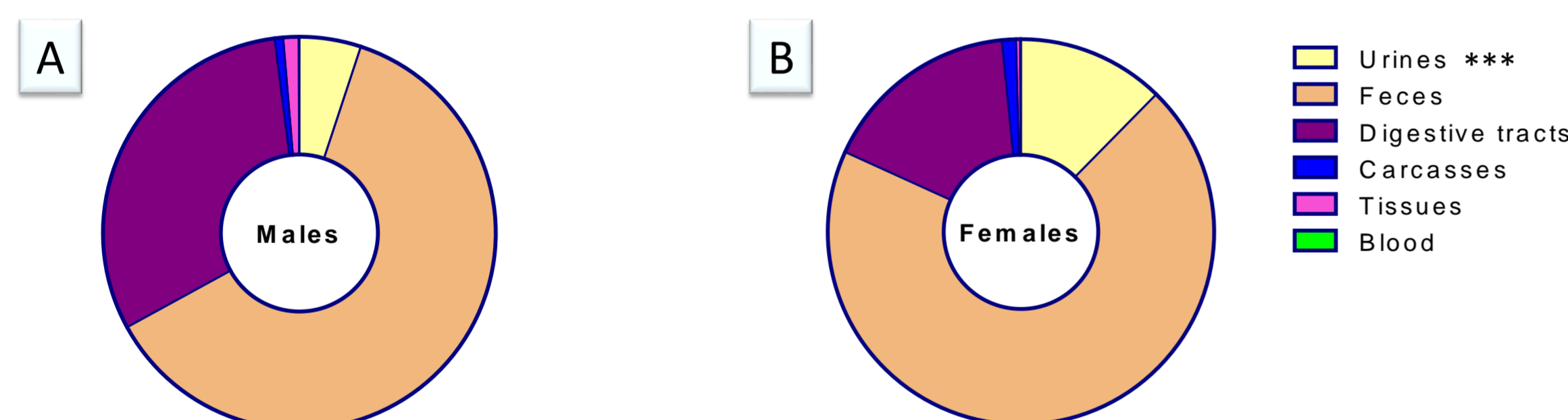


Fig. 1: 24h metabolic balance in male (A) and female (B) Wistar rats. Results (mean, n = 3 for each group) are expressed as % of the administered radioactivity (*t*-test, *** *p*-value ≤ 0.001, Graphpad®)

- 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 **urine**: 4.5 ± 0.9 % recovered in males, 11.8 ± 0.9 % in females.

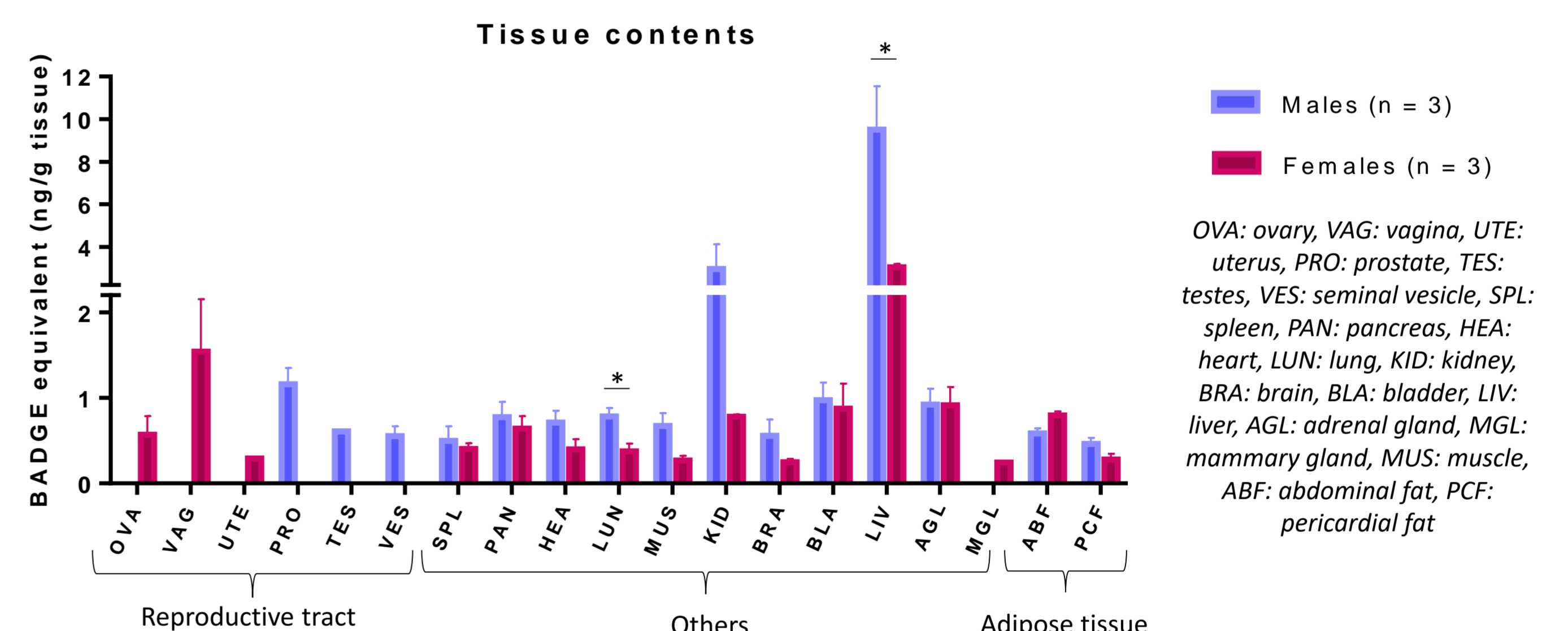


Fig. 3: Residual levels of radioactivity in tissues of male and female Wistar rats. Results (mean ± SEM) are expressed in ng of BADGE equivalent/g tissue (ppb) (*t*-test, * *p*-value ≤ 0.05, Graphpad®)

Digestive tract contents

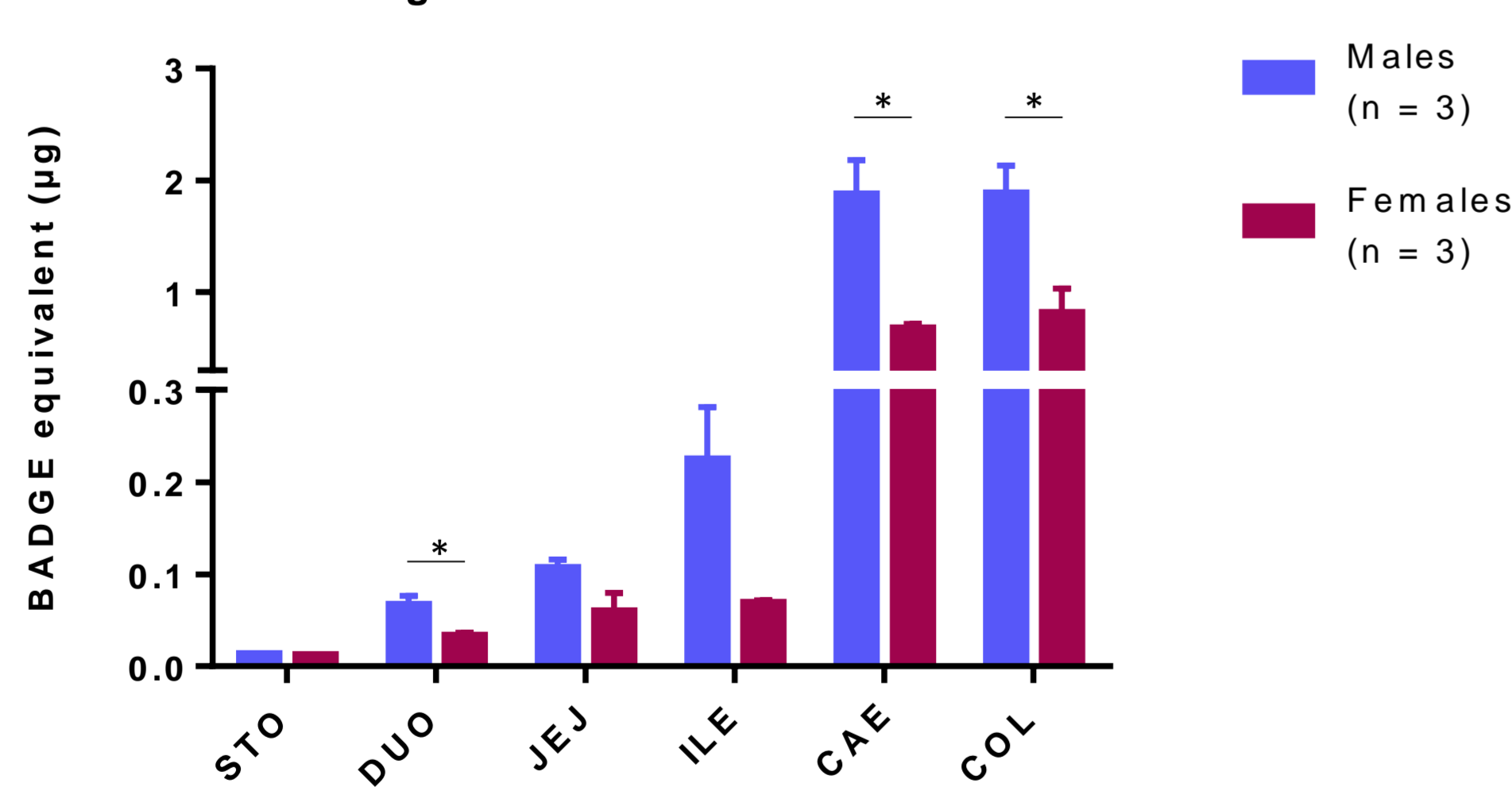


Fig. 2: 24h metabolic balance: residual radioactivity in digestive tract contents of male and female Wistar rats. Results (mean ± SEM) are expressed in µg of BADGE equivalent (*t*-test, * *p*-value ≤ 0.05, Graphpad®)

STO: stomach, DUO: duodenum, JEJ: jejunum, ILE: ileon, CAE: caecum, COL: colon

Radiochromatographic profiling of urine and feces

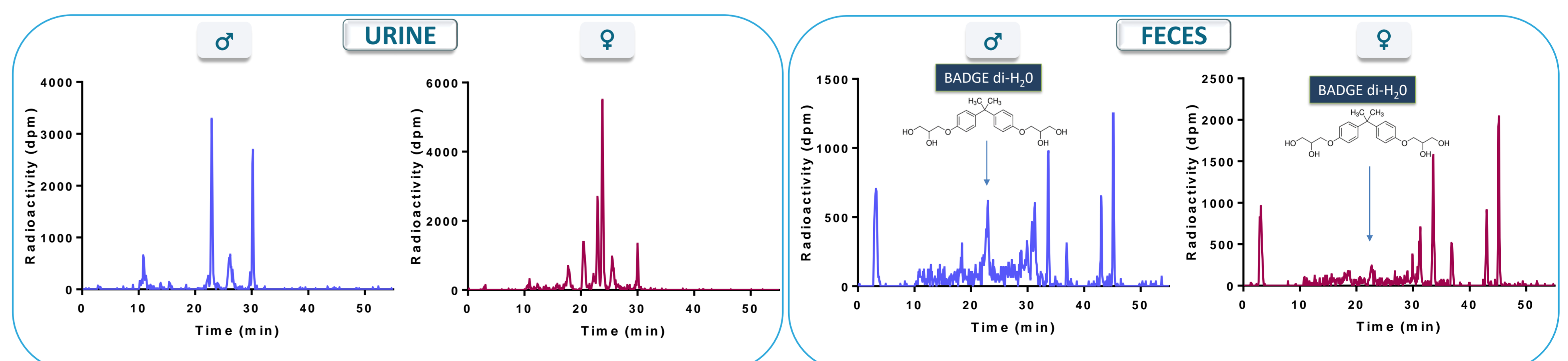


Fig. 4: Typical Radio-HPLC profiles for urine and feces collected over 24h

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

Conclusions

In this **first *in vivo* 24h-study** of [³H]-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

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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)