

# Successful Use of [<sup>14</sup>C]Paracetamol Microdosing to Elucidate Developmental Changes in Drug Metabolism

Miriam G. Mooij<sup>1</sup> · Esther van Duijn<sup>4</sup> · Catherijne A. J. Knibbe<sup>5,6</sup> ·  
Karel Allegaert<sup>1,7</sup> · Albert D. Windhorst<sup>8</sup> · Joost van Rosmalen<sup>3</sup> · N. Harry Hendrikse<sup>9</sup> ·  
Dick Tibboel<sup>1</sup> · Wouter H. J. Vaes<sup>4</sup> · Saskia N. de Wildt<sup>1,2</sup>

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## Abstract

**Background** We previously showed the practical and ethical feasibility of using [<sup>14</sup>C]-microdosing for pharmacokinetic studies in children. We now aimed to show that this approach can be used to elucidate developmental changes in drug metabolism, more specifically, glucuronidation and sulfation, using [<sup>14</sup>C]paracetamol (AAP). **Methods** Infants admitted to the intensive care unit received a single oral [<sup>14</sup>C]AAP microdose while receiving intravenous therapeutic AAP every 6 h. [<sup>14</sup>C]AAP pharmacokinetic parameters were estimated. [<sup>14</sup>C]AAP and metabolites were measured with accelerator mass spectrometry. The plasma area under the concentration-time curve from time zero to infinity and urinary recovery ratios were related to age as surrogate markers of metabolism.

**Results** Fifty children [median age 6 months (range 3 days–6.9 years)] received a microdose (3.3 [2.0–3.5] ng/kg; 64 [41–71] Bq/kg). Plasma [<sup>14</sup>C]AAP apparent total clearance was 0.4 (0.1–2.6) L/h/kg, apparent volume of distribution was 1.7 (0.9–8.2) L/kg, and the half-life was 2.8 (1–7) h. With increasing age, plasma and urinary AAP-glu/AAP and AAP-glu/AAP-sul ratios significantly increased by four fold, while the AAP-sul/AAP ratio significantly decreased.

**Conclusion** Using [<sup>14</sup>C]labeled microdosing, the effect of age on orally administered AAP metabolism was successfully elucidated in both plasma and urine. With minimal burden and risk, microdosing is attractive to study developmental changes in drug disposition in children.

✉ Saskia N. de Wildt  
saskia.dewildt@radboudumc.nl

<sup>1</sup> Intensive Care and Department of Pediatric Surgery, Erasmus MC-Sophia Children's Hospital, Rotterdam, The Netherlands

<sup>2</sup> Department of Pharmacology and Toxicology, Radboud University, PO box 9101, Geert Grooteplein 21, Nijmegen 6500 HB, The Netherlands

<sup>3</sup> Department of Biostatistics, Erasmus MC, Rotterdam, The Netherlands

<sup>4</sup> TNO, Zeist, The Netherlands

<sup>5</sup> Division of Pharmacology, Faculty of Science, Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands

<sup>6</sup> Department of Clinical Pharmacy, St Antonius Hospital, Nieuwegein, The Netherlands

<sup>7</sup> Department of Development and Regeneration, KU Leuven, Louvain, Belgium

<sup>8</sup> Department of Radiology and Nuclear Medicine, VU University Medical Center, Amsterdam, The Netherlands

<sup>9</sup> Department of Pharmacy and Clinical Pharmacology, VU University Medical Center, Amsterdam, The Netherlands

## Key Points

Information gaps still remain on drug metabolism in children, especially on phase II metabolism. Drug metabolism phenotyping studies in children present ethical challenges inherent to risks related to potential toxic effects of the phenotyping probe.

[<sup>14</sup>C]-Microdosing is feasible to phenotype drug metabolism in children. We show a significant decrease in the relative extent of paracetamol sulfation and confirm an increase in glucuronidation in the first 6 years of life after a single oral dose administration.

Microdosing appears a promising tool to overcome ethical barriers to study the ontogeny of drug metabolism, and potentially, also other drug disposition pathways in children. This may aid to a better understanding of developmental pharmacology to enable age-appropriate dosing for ultimately effective and safe pharmacotherapy.

## 1 Introduction

Drug disposition in children may differ largely from that in adults owing to age-related changes in drug absorption, distribution, metabolism, and excretion [1, 2]. Drug metabolism is one of the main determinants of variation in drug disposition. While several knowledge gaps on the ontogeny of drug-metabolizing enzymes have already been elucidated (e.g., cytochrome P450 3A family) much is still unknown, especially on the ‘phase II’ drug-metabolizing enzymes [3, 4].

Phenotyping studies can be used to elucidate *in vivo* drug metabolism pathways but in the pediatric population these studies pose several challenges. For one, when a validated phenotype probe for the pathway of interest is available [5], it is often a marketed drug with potentially therapeutic and toxic effects, which may not be acceptable to ethics committees, physicians, parents, and their children. Second, repeated blood sampling for pharmacokinetic analyses may be difficult in light of blood volume limits (<5% of estimated circulating volume) and repeated painful punctures. The alternative method, urinary sampling, is technically challenging in young children especially without a urinary catheter and results often in unreliable collections.

To overcome the risk associated with therapeutic or toxic effects of a regular phenotype probe, microdosing with carbon-14-labeled drugs may be an interesting

alternative [6–8]. A very low drug dose is not expected to be toxic or result in any therapeutic effect. Additionally, only very small blood volumes are needed for highly sensitive analytical methods, such as accelerator mass spectrometry (AMS) [9]. A microdose is defined as a maximum of 100 µg or 1/100th of the no observed adverse effect level or animal-based predicted pharmacologic dose [10, 11]. Microdosing has been accepted by the US Food and Drug Administration and European Medicines Agency as a tool to study pharmacokinetics in adults [9]. If pharmacokinetics show dose-linear behavior, pharmacokinetic data can be extrapolated to therapeutic exposure [12–14]. Moreover, when a labeled oral dose is given simultaneously with a therapeutic intravenous dose (or routes reversed), bioavailability can easily be determined, on the condition that gastrointestinal dissolution, absorption, and first-pass metabolism is proportional. The microdose is then referred to as a microtracer. Two recent proof-of-concept studies in a limited number of patients have shown the ethical and practical feasibility of pediatric microdosing/microtracer studies using [<sup>14</sup>C]paracetamol [13, 14]. Dose linearity was shown in adults for paracetamol (AAP) from microdose to literature-reported therapeutic dose under standard and inhibition conditions [15, 16]. Our own proof-of-concept study supported the dose linearity of AAP pharmacokinetics in children [13].

Paracetamol, also known as acetaminophen or *N*-acetyl-*p*-aminophenol, is a common analgesic and antipyretic agent. Its main metabolism pathways are glucuronidation and sulfation. Glucuronidation occurs mainly via uridine 5'-diphospho-glucuronosyltransferase (UGT) 1A1, 1A6, 1A9, and 2B15 and sulfation is catalyzed by sulfotransferase (SULT) 1A1, 1A3, 1A4, and 2A1 [17]. Although not specific for individual enzymes, AAP pharmacokinetic studies have provided valuable insight into the relative contributions of glucuronidation and sulfation to the disposition of AAP. Paracetamol metabolism in young children differs from that in adults. In neonates, a higher proportion of urinary paracetamol sulfate than glucuronide suggested immaturity of the involved UGTs, compensated for by more mature sulfotransferases [18]. Other studies showed a significant increase in the urinary paracetamol-glucuronide (AAP-glu)/paracetamol-sulfate (AAP-sul) ratio from 0.27 in 23-week-old preterm neonates to 0.75 in 3- to 9-year-old children and 2 in adults [19].

Despite these studies, gaps remain in the knowledge on the ontogeny of AAP metabolism, especially for children with bodyweights between 3 and 7 kg as well as between 25 and 50 kg [20, 21]. Most developmental changes in drug-metabolizing enzymes occur in the first months of life. Hence, a better insight into the exact pattern of the age-related change in AAP metabolism may provide valuable novel information. Moreover, studies on exclusive

oral AAP and metabolite disposition are scarce, while population pharmacokinetic studies using multiple cohorts have often combined administration routes [20–22]. The bioavailability of orally and rectally administered drugs differs, hence phenotyping of combined small intestinal and hepatic drug metabolism may be less precise [23]. Even though the oral bioavailability of AAP approaches 90% in adults, data in children are lacking and therefore a contribution of intestinal metabolism on oral AAP disposition cannot be excluded (Tylenol<sup>®</sup> Johnson & Johnson, New Brunswick N.J., product information). Finally, most studies used the urinary metabolite ratio as surrogate marker for AAP metabolism. This method has limitations owing to the difficult nature of complete and prolonged urine collections in young children, with incomplete or short collection intervals, and may have unexplained large inter-individual variation reducing the power to detect more subtle age-related variation.

Previously, we have shown that microdosing is practically and ethically feasible to study AAP pharmacokinetics in children [13]. Now, using this innovative [<sup>14</sup>C]-microdosing method, we aim to study the impact of age on the disposition of orally administered AAP, over the age range of 0–6 years. Plasma and urine analyses include metabolites (AAP-glu, AAP-sul).

## 2 Methods

### 2.1 Subjects

Patients admitted to the pediatric intensive care unit of the Erasmus MC-Sophia Children's Hospital were considered for inclusion. Inclusion criteria were age from 0 through 6 years, medical need for intravenous AAP, an indwelling arterial or central venous line in place, and written informed consent from parents or legal guardians. To reduce the impact of critical illness or bowel disease on the pharmacokinetics of AAP, patients with kidney and liver failure, more than one vasopressor drug, inflammatory bowel disease, or who were intolerant to enteral nutrition, or concomitantly receiving co-medication known to interact with AAP (according to Micromedex<sup>®</sup>, Truven Health Analytics Inc., Michigan, [www.micromedexsolutions.com](http://www.micromedexsolutions.com)) were excluded.

### 2.2 Study Design

The study design has been previously described in detail [13]. The Dutch Central Committee on Research Involving Human Subjects (The Hague) approved the study (EudraCT 2011-005497-28). Purchase of [<sup>14</sup>C]AAP formulation and radiopharmaceutical preparation have been

previously described [13]. A single [<sup>14</sup>C]AAP microdose (3.3 ng/kg, 60 Bq/kg, 0.25 mL/kg) was given by mouth or enteral feeding tube. The Dutch Nuclear Research and Service Group estimated the radiation exposure of a single microdose at <1 μSv and the exposure was explained to parents/caregivers in relation to a continental flight (>4 μSv), medical imaging, or background radiation (2.5 μSv/year in The Netherlands) [24]. The [<sup>14</sup>C]AAP oral dose also contained 1.7 μg/kg non-labeled AAP, which is negligible in relation to the therapeutic intravenous dose and considered irrelevant for pharmacokinetic estimations. Enteral feeding tubes were flushed with 2 mL of saline to ensure complete rinsing. Patients would receive the [<sup>14</sup>C]AAP microdose as soon as the pump with the therapeutic intravenous AAP was started as part of clinical care, dosed according to the *Dutch Pediatric Handbook* (<http://www.kinderformularium.nl>): a 20-mg/kg loading dose, followed by 10 mg/kg every 6 h (<1 month of age) or 15 mg/kg every 6 h (≥1 month of age). The oral [<sup>14</sup>C]AAP microdose would be given with the first or a sequential AAP intravenous dose.

Blood samples (1.0 mL) were taken from the indwelling catheter immediately before administration; at around 10 and 30 min; and at 1, 2, 4, 6, 12, and 24 h after dosing or until the catheter was removed. After centrifuging, plasma was stored at –80 °C until analysis. Urine was collected in patients who needed a urinary catheter for clinical reasons. It was collected for a maximum 24 h or until the urinary catheter was removed. Urine volume was entered by the nurses in the clinical electronic patient record. One sample (2 mL) was taken from the 24-h urine collection and stored at –80 °C until analysis.

### 2.3 Analytical Procedures

Previously, a method has been qualified to quantify [<sup>14</sup>C]AAP, [<sup>14</sup>C]AAP-glu, and [<sup>14</sup>C]AAP-sul levels in human plasma, using liquid chromatography in combination with AMS [13, 25]. The liquid chromatography plus AMS qualification was performed in accordance with the recommendation of the European Bioanalytical Forum [26]. Qualification results are summarized in Mooij et al. [13].

In short, the experimental outline was as follows; a 45-μL aliquot of plasma was extracted using 175 μL methanol containing 6.6 μg/mL AAP, 8.10 μg/mL AAP-glu, 10.0 μg/mL AAP-sul, and 6.6 μg/mL 3-acetamidophenol in 96-well protein precipitation plates. The pellet was washed with 100 μL 0.9% NaCl: 100% methanol (1:4 v/v). The filtrates were combined and after evaporation, redissolved in 30 μL 10 mM ammonium phosphate pH 3.4 (Eluent A). For each sample, 25 μL was injected on the ultra performance liquid chromatography. Calibration

**Table 1** Patient demographics

	Age group 1 (birth–1 month)	Age group 2 (1 month–6 months)	Age group 3 (6 months–2 years)	Age group 4 (2 years–6 years)
Number	9	15	14	12
Postnatal age in weeks	0 (0–1)	23 (7–25)	35 (26–98)	197 (112–361)
Gestational age at birth in weeks	38.1 (34.9–40.7)	39.8 (33.1–42)	39.9 (30–41.7)	40 (36–40)
Weight (kg)	3 (2–3.5)	7 (3.5–9.2)	7.5 (6.1–12.8)	16.1 (12.9–25)
Weight (z-score)	–1.12 (–2.1 to 0.1)	–0.63 (–2.5 to 1.5)	–1.3 (–2.5 to 0.9)	–0.02 (–2.9 to 2.1)
Gender (M/F)	8/1	14/1	10/4	9/3
Ethnicity (Caucasian/African/Asian/Other)	5/1/2/1	11/1/1/2	12/0/0/2	11/0/0/1
Reason for admission (surgical/medical)	8/1	12/3	12/2	6/6
Mechanical ventilation at start study (yes/no)	1/8	0/15	0/14	0/12
Enteral/tube feeding (yes/no)	3/6	10/5	9/5	6/6
PELOD score (on the study day)	11 (0–11)	10 (0–30)	6.5 (0–30)	10 (1–20)
Number of organs with dysfunction (on the study day)	2 (0–2)	1 (0–3)	2 (0–3)	1 (1–3)
PRISM score	16 (0–31)	18 (4–41)	17 (0–34)	6 (0–44)
PIM score (%)	4 (0–20)	1 (0–10)	0 (0–9)	2 (0–33)
PIM II score (%)	5 (1–15)	0 (0–17)	0 (0–6)	1 (0–29)
<b>Paracetamol dosing</b>				
[ <sup>14</sup> C]AAP dose (ng)	9.8 (6.5–11.7)	22.8 (11.7–29.9)	23.4 (15.6–41.6)	52 (41.6–81.2)
[ <sup>14</sup> C]AAP dose (Bq)	209 (125–228)	472 (235–639)	477 (313–890)	1035 (833–1594)
[ <sup>14</sup> C]AAP dose normalized (mg/kg) (dose × 5E6)/weight)	15.6 (16.3–17.3)	16.2 (16.07–17)	16.3 (10–16.7)	16.3 (16.1–16.7)
Microdose administration via (oral/gastric tube/duodenal tube/gastrostomy)	0/6/1/0	6/4/2/0	3/6/1/0	1/3/4/2

## Medians and ranges

AAP paracetamol, PELOD pediatric logistic organ dysfunction, *Number of organs with dysfunction* numbers of organs scoring at least one in the PELOD score, PRISM pediatric risk of mortality, PIM pediatric index of mortality

standards at eight different levels, ranging from 0.4 to 180 Bq/L were prepared by spiking an [<sup>14</sup>C]AAP solution (SA 4100 Bq [<sup>14</sup>C]AAP/100 µg AAP) to blank pooled plasma. Similarly, quality standards were prepared at three levels, from 1.7 to 131 Bq/L. For the calibration and quality standards, only 15 µL of the sample was used for extraction. Chromatographic conditions can be found in Table 1 of Mooij et al. [13]. The extraction recovery per sample was determined by the area under the concentration-time curve (AUC) of spiked AAP, AAP-glu, and AAP-sul vs. the AUC of the corresponding compounds after a direct injection of the extraction solution. The collected fractions of AAP, AAP-glu, and AAP-sul were supplemented with 25 µg carbon-12, by adding 25 µL 1.97 mg/mL AAP. Fractions were transferred to a tin foil cup and evaporated to dryness prior to AMS analysis.

Urine samples were processed similarly, with a few exceptions. Urine samples were diluted 20-fold with MilliQ water prior to extraction. Like for plasma samples, 45 µL of the 20-fold diluted urine samples was extracted. The extraction solution was spiked with 6.6 µg/mL

3-acetamidophenol only, which was used as an internal standard to determine the recovery of the extraction. As relatively high concentrations of cold AAP, AAP-glu, and AAP-sul were present in urine samples owing to the therapeutic dose the patients received; recoveries could not be determined from spiked AAP, AAP-glu, and AAP-sul. Each analytical run included the duplicate analysis of the calibrators and triplicate analysis of quality standards, and passed based on the acceptance criteria [26].

Tin foil cups were combusted on an elemental analyzer (Vario Micro; Elementar, Langenselbold, Germany). Generated CO<sub>2</sub> was transferred to a home-built gas interface, composed of a zeolite trap and syringe [25]. CO<sub>2</sub> adsorbed to the trap on the interface; after heating of the trap, the CO<sub>2</sub> was transferred to a vacuum syringe using helium. A final CO<sub>2</sub>/helium mixture of 6% was directed to the AMS ion source, at a pressure of 1 bar and a flow of 60 µL/min. A 1-MV Tandetron AMS (High Voltage Engineering Europe B.V., Amersfoort, The Netherlands) [27] was used. The lower limit of quantification (LLOQ) of the method was 0.58 Bq/L.

## 2.4 Pharmacokinetic Analysis

The maximal concentration of [<sup>14</sup>C]AAP in plasma ( $C_{\max}$ ) and time to reach  $C_{\max}$  ( $T_{\max}$ ) were extracted from the plasma concentration vs. time curve. The AUC from time zero to the last sampling time point ( $AUC_{0-t}$ ) was calculated using the log-linear trapezoidal method. The samples below the LLOQ were set on 0.29 Bq/L ( $1/2 \times$  LLOQ). The apparent volume of distribution ( $V_{ss}$ ) and total plasma clearance (CL) were calculated by using standard non-compartmental techniques. Pharmacokinetic parameters of [<sup>14</sup>C]AAP-glu and [<sup>14</sup>C]AAP-sul were determined as described above for [<sup>14</sup>C]AAP. All pharmacokinetic parameters derived from individual patients were estimated using the Excel PKsolver add-in software [28].

The plasma AUC from time zero to infinity ( $AUC_{0-\infty}$ ) and urinary recovery ratios of [<sup>14</sup>C]AAP-glu/[<sup>14</sup>C]AAP, [<sup>14</sup>C]AAP-sul/[<sup>14</sup>C]AAP, and [<sup>14</sup>C]AAP-glu/[<sup>14</sup>C]AAP-sul were calculated to serve as ‘surrogate’ markers for respectively glucuronidation and sulfation and their relative activity. Because AUC ratios were used for the primary outcomes, individual AUCs were not corrected for individual dose. For clarity, the  $AUC_{0-\infty}$  ratios of [<sup>14</sup>C]AAP-glu/[<sup>14</sup>C]AAP are denoted as ‘AAP-glu/AAP’ and the urinary recovery ratios are denoted as ‘U-AAP-glu/U-AAP’.

Criteria for excluding study patients from all pharmacokinetic analyses were inadequate intake or too few blood samples (fewer than four). If  $CL/F$ ,  $V_{ss}/F$ , or half-life ( $t_{1/2}$ ) were not predicted because of non-negative estimated  $\lambda_z$ , they were excluded for respectively  $CL/F$ ,  $V_{ss}/F$ , or  $t_{1/2}$  analyses. If the part of  $AUC_{0-\infty}$  that was extrapolated beyond the last observation was larger than 20% of the actual  $AUC_{0-t}$ , then the  $AUC_{0-\infty}$  was excluded from the analyses, as it would introduce unreliable overestimation of the  $AUC_{0-\infty}$ .

## 2.5 Statistics

The [<sup>14</sup>C]AAP and metabolite concentrations were measured using Bq/L and converted to ng/L based on the exact dose given (3.3 ng/kg contained 60 Bq/kg). For the metabolites,  $C_{\max}$  was corrected for molecular weight (AAP-glu/AAP 2.165 and AAP-sul/AAP 1.530). To compare the disposition of oral [<sup>14</sup>C]AAP microdose to literature data of therapeutically dosed AAP, the microdose concentrations were dose normalized to 15 mg/kg (assuming bioavailability is dose linear) by multiplying the plasma [<sup>14</sup>C]AAP and metabolite concentrations (in ng/L) by  $5 \times 10^6$  [29].

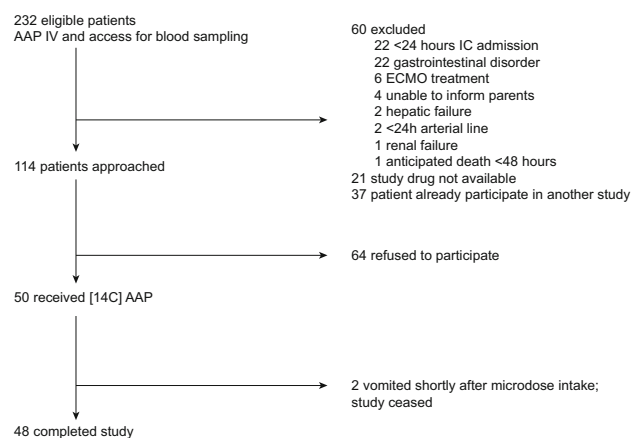
Descriptive statistics of all variables are presented as medians and ranges. Nonparametric Spearman’s rank correlation was used to describe the relationship between

plasma and urinary metabolite ratios. Univariable linear regression analyses were performed with postnatal age as an independent variable and the pharmacokinetic parameters ( $C_{\max}$ ,  $T_{\max}$ ,  $t_{1/2}$ ,  $AUC_{0-\infty}$ , weight-corrected  $CL/F$ , and weight-corrected  $V_{ss}/F$ ), including the AUC ratios as dependent variables. If necessary, dependent and independent variables were log-transformed to ensure normality of the residuals and an adequate model fit. The  $T_{\max}$  was compared between the different modes of oral administration (oral, gastric/duodenal tube, or gastrostomy) using the Kruskal–Wallis test. All statistical tests were two-sided and used a significance level of  $p = 0.05$ .

## 3 Results

### 3.1 Patients

From 13 January, 2014 till 1 July, 2015, 50 patients (41 boys) with a median gestational age at birth of 40 (30–42) weeks, and a median postnatal age of 6 months (3 days till 6 years and 11 months) participated in the study (Fig. 1; Table 1). The main reason for admission was postsurgical care (38 out of 50 patients). One patient needed mechanical ventilation. Patients received one oral [<sup>14</sup>C]AAP microdose (median [range]: 3.3 [2.0–3.5] ng/kg; 64 [41–71] Bq/kg) and up to four intravenous AAP doses during the 24-h study period as per clinical protocol. Variation in dose was introduced by round-off errors and in one case a body-weight error was found in the health record and rectified after microdose administration leading to a dose of 2.0 ng/kg, 41 Bq/kg. In some cases, the route of administration was switched from intravenous to rectal for clinical reasons after the microdose was given. In two cases, the blood sampling was discontinued and excluded from the



**Fig. 1** Flow chart of patient recruitment. AAP paracetamol, IC intensive care, IV intravenous, ECMO extracorporeal membrane oxygenation



pharmacokinetics analysis because the patient vomited shortly after administration of the microdose, potentially resulting in inadequate [ $^{14}\text{C}$ ]AAP intake. For nine patients, the number of blood samples taken was too small (fewer than four) to accurately estimate all pharmacokinetic parameters and these patients were excluded from the pharmacokinetics analysis. Of the 39 patients for the [ $^{14}\text{C}$ ]AAP pharmacokinetics analysis, 19 received the microdose [ $^{14}\text{C}$ ]AAP via a gastric tube, ten via oral administration (in a buccal cavity), eight via a duodenal tube, and two via gastrostomy. Three patients received potentially interacting co-medication (phenytoin, phenobarbital, and pentobarbital) after administration of the study drug for clinical reasons. For an additional patient,  $\text{AUC}_{0-t}$ ,  $C_{\text{max}}$ , and  $T_{\text{max}}$  could be estimated but not  $\text{CL}/F$ ,  $V_{\text{ss}}/F$ , and  $t_{1/2}$ .

### 3.2 Pharmacokinetics

The exemplary plasma concentration-time curves for [ $^{14}\text{C}$ ]AAP, [ $^{14}\text{C}$ ]AAP-glu, and [ $^{14}\text{C}$ ]AAP-sul of two individual patients are depicted in Fig. 2. The pharmacokinetic parameters are summarized in Table 2. The number of samples below LLOQ were respectively 20 (8%), 41 (16%), and 33 (13%) for [ $^{14}\text{C}$ ]AAP, [ $^{14}\text{C}$ ]AAP-glu, and [ $^{14}\text{C}$ ]AAP-sul of a total of 256 samples.  $T_{\text{max}}$  for [ $^{14}\text{C}$ ]AAP was similar for all routes of administration, but with large variability (Table 2).  $C_{\text{max}}$ , weight-corrected  $\text{CL}/F$ , and  $V_{\text{ss}}/F$  were also similar for all routes of administration and similar between buccal and other administrations (gastric tube, duodenal tube, or gastrostomy).

Dose-normalized ( $\text{mg}/\text{kg}$  dose)  $C_{\text{max}}$  for [ $^{14}\text{C}$ ]AAP, [ $^{14}\text{C}$ ]AAP-glu, and [ $^{14}\text{C}$ ]AAP-sul were respectively, 8.4 (0.9–23.0)  $\text{mg}/\text{L}$ , 3.8 (0.4–16.0)  $\text{mg}/\text{L}$ , and 4.6 (0.5–20.5)  $\text{mg}/\text{L}$ . One outlier could be identified, but was kept in the analysis; this patient had very low plasma [ $^{14}\text{C}$ ]AAP and metabolite concentrations, even though no abnormalities were noticed during oral dose administration. The patient may have partially spit out the buccally given microdose.

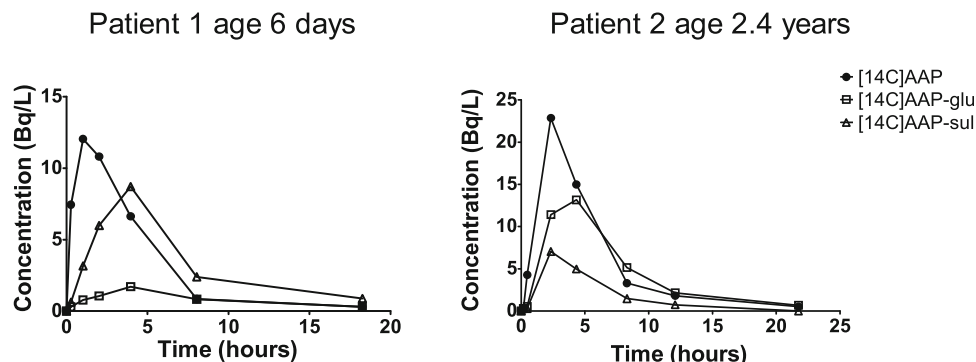
### 3.3 Effect of Age on [ $^{14}\text{C}$ ]AAP Pharmacokinetics and Metabolism

No significant relationship was detected between postnatal age (which was log-transformed) and [ $^{14}\text{C}$ ]AAP  $C_{\text{max}}$ ,  $T_{\text{max}}$ ,  $t_{1/2}$ ,  $\text{AUC}_{0-\text{inf}}$ , weight-corrected  $\text{CL}/F$ , or weight-corrected  $V_{\text{ss}}/F$ . The AAP-glu/AAP ratio increased with postnatal age ( $n = 35$ ,  $R^2 = 0.223$ ,  $p < 0.01$ ) (Fig. 3a), whereas the AAP-sul/AAP ratio decreased with postnatal age ( $n = 36$ ,  $R^2 = 0.255$ ,  $p < 0.01$ ) (Fig. 3b). Moreover, the AAP-glu/AAP-sul ratio increased with age from 0.32 at birth to 1.23 at the age of 6 years ( $n = 34$ ,  $R^2 = 0.456$ ,  $p < 0.001$ ) [Fig. 3c]. In these analyses, postnatal age and all plasma and urine ratio outcomes were both log-transformed. No significant differences were found in the AAP-glu/AAP, AAP-sul/AAP, and AAP-glu/AAP-sul ratios between the different routes of administration (respectively,  $p = 0.6$ ,  $p = 0.3$ , and  $p = 0.2$ ).

Urine was collected reliably for at least 12 h (20.4 h [11.8–28.5 h]) in 24 patients. Total median (range) urinary recovery of [ $^{14}\text{C}$ ]AAP plus [ $^{14}\text{C}$ ]AAP-glu and [ $^{14}\text{C}$ ]AAP-sul as the percentage of dose was 75% (28–99%). Recovery of [ $^{14}\text{C}$ ]AAP, [ $^{14}\text{C}$ ]AAP-glu, and [ $^{14}\text{C}$ ]AAP-sul are shown in Table 2. Percentage recovery of [ $^{14}\text{C}$ ]AAP-glu increased with postnatal age ( $n = 24$ ,  $R^2 = 0.306$ ,  $p < 0.01$ ); [ $^{14}\text{C}$ ]AAP-sul recovery decreased with postnatal age ( $n = 24$ ,  $R^2 = 0.175$ ,  $p < 0.05$ ); while [ $^{14}\text{C}$ ]AAP recovery remained stable with postnatal age ( $n = 24$ ,  $R^2 = 0.004$ ,  $p = 0.78$ ). The percentage of total urinary [ $^{14}\text{C}$ ] recovery ([ $^{14}\text{C}$ ]AAP + [ $^{14}\text{C}$ ]AAP-glu + [ $^{14}\text{C}$ ]AAP-sul) was computed for each individual separately. The percentage of total urinary [ $^{14}\text{C}$ ] recovery also did not change with age ( $n = 24$ ,  $R^2 = 0.003$ ,  $p = 0.79$ ).

The U-AAP-glu/U-AAP ratio significantly increased with postnatal age ( $n = 24$ ,  $R^2 = 0.456$ ,  $p < 0.001$ ) (Fig. 4a), whereas the U-AAP-sul/U-AAP ratio showed a borderline non-significant decrease with age ( $n = 24$ ,  $R^2 = 0.150$ ,  $p = 0.06$ ) (Fig. 4b). The U-AAP-glu/U-AAP-sul ratio increased linearly with postnatal age ( $n = 24$ ,  $R^2 = 0.575$ ,  $p < 0.001$ ) (Fig. 4c). A strong positive

**Fig. 2** Two individual plots: plasma concentration-time curve AAP, AAP-glu, and AAP-sul (Bq/L). AAP paracetamol, AAP-glu paracetamol-glucuronide, AAP-sul paracetamol-sulfate



**Table 2** [<sup>14</sup>C]AAP pharmacokinetics

	[ <sup>14</sup> C]AAP	[ <sup>14</sup> C]AAP-glu	[ <sup>14</sup> C]AAP-sul
<b>Plasma</b>			
AUC (0, <i>t</i> ) (Bq/L h)	152 (24–462) ( <i>n</i> = 39)	70 (9–339) ( <i>n</i> = 39)	69 (8–576) ( <i>n</i> = 39)
AUC (0, inf) (Bq/L h)	152 (25–489) ( <i>n</i> = 38 <sup>a</sup> )	75 (11–367) ( <i>n</i> = 35 <sup>a</sup> )	75 (9–597) ( <i>n</i> = 37 <sup>a</sup> )
CL/ <i>F</i> (L/h)	3.1 (0.4–26.6) ( <i>n</i> = 38)	–	–
CL/ <i>F</i> (L/h/kg)	0.4 (0.1–2.6) ( <i>n</i> = 38)	–	–
<i>V</i> <sub>ss</sub> / <i>F</i> (L)	11.1 (3.0–82.2) ( <i>n</i> = 38)	–	–
<i>V</i> <sub>ss</sub> / <i>F</i> (L/kg)	1.7 (0.9–8.2) ( <i>n</i> = 38)	–	–
<i>t</i> <sub>1/2</sub> (h)	2.8 (1.0–7.0) ( <i>n</i> = 38)	–	–
<i>C</i> <sub>max</sub> (Bq/L)	34.9 (3.8–89.6) ( <i>n</i> = 39)	8.6 (0.8–38.1) ( <i>n</i> = 39)	10.0 (1.1–41.0) ( <i>n</i> = 39)
<i>C</i> <sub>max</sub> (ng/L)	1.7 (0.2–4.6) ( <i>n</i> = 39)	0.9 (0.1–4.1) ( <i>n</i> = 39)	0.8 (0.1–3.2) ( <i>n</i> = 39)
<i>T</i> <sub>max</sub> (h)	1.0 (0.2–6.2) ( <i>n</i> = 39)	4.1 (0.8–12.6) ( <i>n</i> = 39)	3.0 (0.3–12.6) ( <i>n</i> = 39)
Oral ( <i>n</i> = 10)	2.0 (0.2–4.5)	–	–
Gastric tube ( <i>n</i> = 19)	1.1 (0.2–7)	–	–
Duodenal tube ( <i>n</i> = 8)	0.5 (0.3–2.4)	–	–
Gastrostomy ( <i>n</i> = 2)	1.5 (0.3–2.7)	–	–
AUC(0, inf) ratio	0.4 (0.1–1.6) ( <i>n</i> = 35)	–	–
AAP-glu/AAP			
AUC(0, inf) ratio	0.5 (0.2–1.4) ( <i>n</i> = 36)	–	–
AAP-sul/AAP			
AUC(0, inf) ratio	1.1 (0.2–2.8) ( <i>n</i> = 34)	–	–
AAP-glu/AAP-sul			
<b>Urine</b>			
Urinary recovery as percentage of [ <sup>14</sup> C]AAP dose (%)	10 (4–17)% ( <i>n</i> = 24)	29 (4–46)% ( <i>n</i> = 24)	34 (12–74)% ( <i>n</i> = 24)
Total urinary recovery as percentage of [ <sup>14</sup> C]AAP dose (%)	75% (28–99%) ( <i>n</i> = 24)		

Medians and ranges

<sup>a</sup> Another 1, 4, and 2 patients were excluded from AUC<sub>0–inf</sub> analyses of respectively [<sup>14</sup>C]AAP, [<sup>14</sup>C]AAP-glu, and [<sup>14</sup>C]AAP-sul, as the part of the AUC<sub>0–inf</sub> that was extrapolated was larger than 20% of the AUC<sub>0–*t*</sub>

correlation was found between the AAP-glu/AAP-sul and U-AAP-glu/U-AAP-sul ratios (*n* = 17, *r* = 0.89, *p* < 0.001) (Fig. 5).

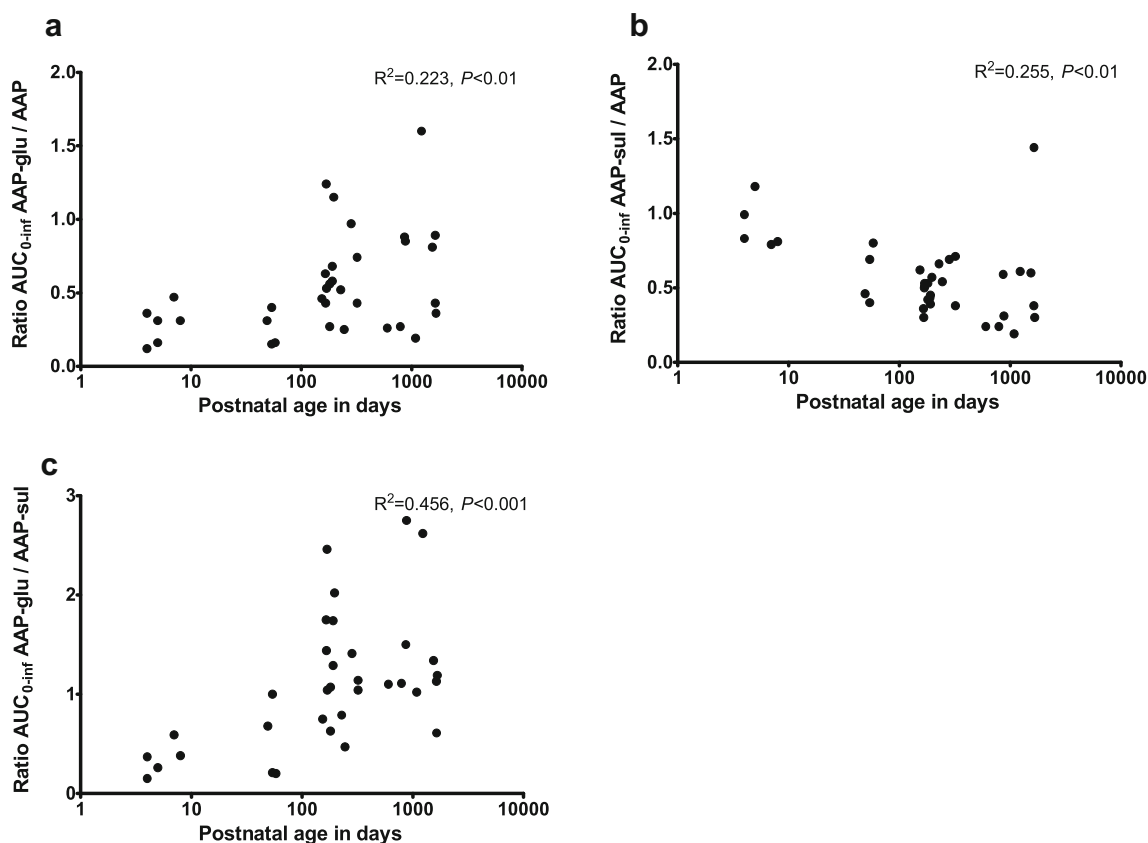
## 4 Discussion

Our study shows that [<sup>14</sup>C]-microdosing allows the studying of age-related changes in drug metabolism in children, using both plasma and urine as a matrix. Both the plasma and the urine [<sup>14</sup>C]AAP glucuronide/sulfate ratios increased approximately four fold with age (from 0 to 6 years), which may reflect the maturation of combined intestinal and hepatic paracetamol glucuronidation and sulfation.

Previously, pediatric studies have reported the AAP pharmacokinetics after oral administration [18, 21, 22, 30–32]. Our data, with average CL/*F* of 0.4 (0.1–2.6) L/h/kg and *V*/*F* of 1.7 (0.9–8.2 L/kg) are largely in line with these studies. In a population pharmacokinetic study combining the data of these studies [31, 33–36],

concerning more than 250 patients (mean age ± standard deviation 11.8 ± 2.5 months) receiving oral and/or rectal AAP, the mean CL/*F*<sub>oral</sub> was 0.2 L/h/kg (95% confidence interval 0.16–0.23) [14.1 L/h/70 kg] and *V*<sub>d</sub>/*F* was 1.12 L/kg (95% confidence interval 1.02–1.21) [78.7 L/70 kg] [21]. CL/*F* was 0.07 L/h/kg and *V*/*F* was 1.088 L/kg in a ‘post-hoc’ NONMEM analysis of a selection of 30 neonates and infants (aged 1–90 days) [22]. The latter study also found an age-related CL/*F* on pooled analyses of 221 children (aged 0–17 years). In contrast to this finding, we did not find an effect of age on weight-corrected CL/*F*, but this might be explained by the small number of neonates in our cohort or the absence of more complex exponential functions to describe our data.

In our study, both the plasma and the urine AAP-glu/AAP-sul ratio were related to age; the plasma ratio increased from 0.32 in neonates to 1.23 in 6-year-old children with a similar increase in urine. The AAP-glu/AAP-sul ratio served as a surrogate marker for the relative contribution of glucuronidation and sulfation to AAP metabolism as in previous studies [18, 19, 21, 37]. Previous



**Fig. 3** **a** Increase of the plasma AAP-glu/AAP ratio with postnatal age. **b** Decrease of the plasma AAP-sul/AAP ratio with increasing age. **c** Increase of the plasma AAP-glu/AAP-sul ratio with postnatal age. For linear regression analysis: plasma ratio and age were both

log-transformed. *AAP* paracetamol, *AAP-glu* paracetamol-glucuronide, *AAP-sul* paracetamol-sulfate,  $AUC_{0-inf}$  area under the concentration-time curve from time zero to infinity

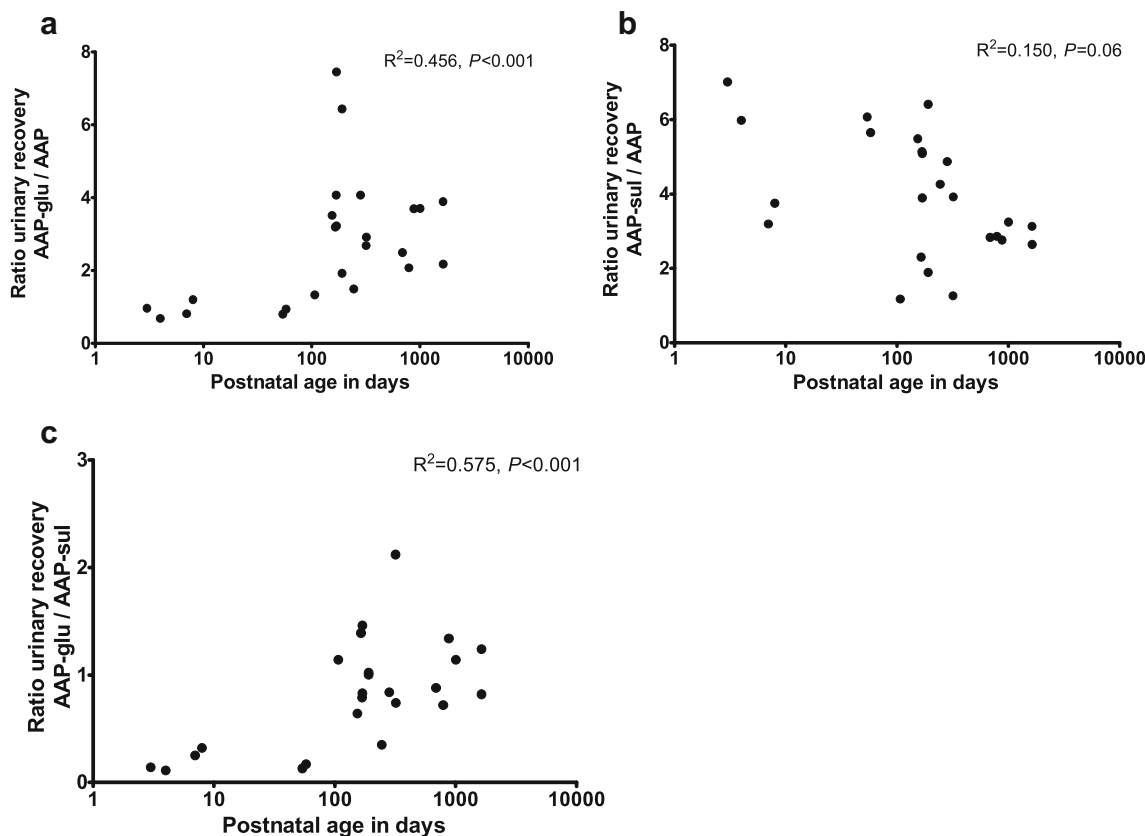
studies reported changes in the urinary metabolites ratio after oral AAP administration from 0.27 in 2- to 3-day-old neonates, to 0.69 in 12-month-old infants, 0.81 in 2-year-old infants, 0.75 in 3- to 9-year-old children, 1.61 in 12-year-old children to 1.8 in adults [18, 21, 30]. Limitations of existing studies were: only reporting neonatal data [30], or few patients aged less than 9 years [18] and a combined cohort of oral and rectal administration, with urinary data on AAP and metabolites (instead of plasma) of 15 patients with a small age range ( $11.8 \pm 2.5$  months) [21]. To the latter study, an additional cohort of >200 children receiving oral and/or rectal AAP were added in the AAP clearance analysis (without metabolite concentrations). However, rectal AAP completely bypasses the small intestine and partially bypasses the liver by draining in the lower hemorrhoidal circulation. Consequently, metabolism of rectally administered AAP does not optimally represent the combined intestinal and hepatic glucuronidation and sulfation activity.

Allegaert et al. showed in 23 preterm and term newborns a significant increase of the urinary glucuronide/total drug ratio with both increasing postnatal and postconceptional

age after intravenous administration of the pro-drug propacetamol [38]. In 75 children (three neonates, 25 infants, 25 children, and 22 adolescents) who received repeated intravenous AAP, glucuronidation was the primary pathway of elimination in the older, but not the neonatal age group, where sulfation dominated [39]. The urinary AAP-glu/AAP-sul ratio collected over 4 h after the last dose, was low in neonates and higher at older age; 0.6 in neonates, 1.0 in infants, 1.4 in children, and 1.2 in adolescents. First-pass drug metabolism after oral drug administration may occur at both the intestine and the liver. Here, we see similar ratio AAP-glu/AAP-sul data after oral (our study) and intravenous administration (Zuppa et al., Allegaert et al.), which suggests that the intestinal contribution to AAP metabolism or the ontogeny of intestinal AAP metabolism either equals hepatic metabolism or is negligible.

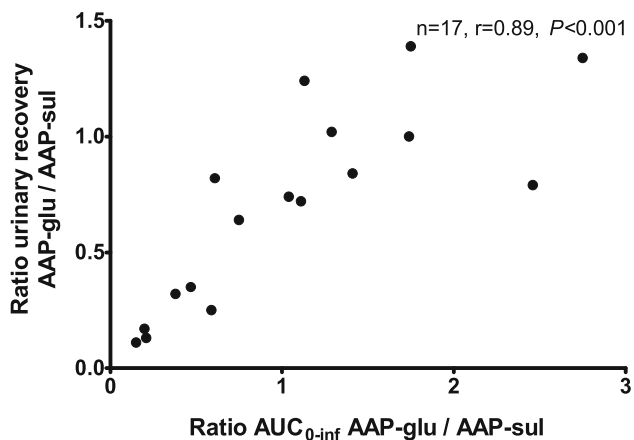
Exploring the maturation  $t_{1/2}$  of AAP-glu and AAP-sul activities (Fig. 3), we modeled a sigmoidal  $E_{max}$  curve for the AAP-glu/AAP-sul ratio (results not shown). The wide confidence intervals of the estimated parameters suggested low evidence for a sigmoidal relationship. Visual inspection of the





**Fig. 4** **a** Increase of the urinary recovery ratio AAP-glu/AAP with postnatal age. **b** Urinary recovery ratio AAP-sul/AAP with postnatal age. **c** Increase of the urinary recovery ratio AAP-glu/AAP-sul with

postnatal age. For linear regression analysis: urine ratio and age were both log-transformed. AAP paracetamol, AAP-glu paracetamol-glucuronide, AAP-sul paracetamol-sulfate



**Fig. 5** Correlation of the plasma AAP-glu/AAP-sul ratio and urinary U-AAP-glu/U-AAP-sul ratio. AAP paracetamol, AAP-glu paracetamol-glucuronide, AAP-sul paracetamol-sulfate, AUC<sub>0-∞</sub> area under the concentration-time curve from time zero to infinity

maturation curves suggests a quite sudden surge in glucuronidation after 200 days of age, but a more gradual decrease in AAP sulfation from birth to 6 years of age.

Genetic polymorphisms are potentially an important factor to explain inter-individual variability. A recent

review proposed several UGT polymorphisms that may affect AAP disposition. To date, especially in children, no polymorphism has proven to be clinically significant [40].

This study has several strengths. First, we show the feasibility of a drug metabolism phenotyping study using [<sup>14</sup>C]AAP with only 0.2 millionth of the therapeutic dose. Although such a design has been used in adults, we now show the feasibility to include a considerable number of pediatric patients in the intensive care unit with a rich blood sampling design and reliable urine collections. In addition, this study provides cross-sectional data on both the plasma and the urinary [<sup>14</sup>C]AAP metabolism after a single oral dose administration in a continuous cohort from 3 days to 6 years of age. These data add to our understanding of the combined intestinal and hepatic glucuronidation and sulfation activities, which is of importance given that most drugs are given orally. Another strength is the combination of urine and plasma collection, showing the feasibility of [<sup>14</sup>C]-microdosing to use either matrix for drug metabolism phenotyping. The similar developmental changes in plasma (this study) and urine [30] suggest that both matrices can be used to study AAP disposition. Plasma calculations were more reliable

( $n = 39$  complete plasma collections vs.  $n = 24$  complete urine collections) with less variation compared with the urinary recovery, but blood sampling is more invasive. Hence, depending on the study population (with/without indwelling arterial/venous access), age range, or renal clearance-dependent drug, both plasma and urine could be considered.

A possible barrier for investigators to use a [ $^{14}\text{C}$ ]-microdosing study in children is the perceived risk of radiation [7]. To our own surprise, in this non-therapeutic study with a radioactive-labeled probe, fear of radiation was scarcely a reason for parents to withhold consent. Even though drug studies in critically ill children in general are challenging in obtaining parental consent [41], similar to other studies on our intensive care unit, almost half of all approached parents agreed to participate [42, 43]. Refusal of consent was rather for reasons relating to the burden of blood sampling or additional procedures. The PAMPER consortium, also using [ $^{14}\text{C}$ ]AAP, experienced the same inclusion rate [14].

A limitation of our study was that study patients did not receive a single microdose exclusively and therefore one could argue that it is not a 'true' microdosing study but a microtracer study. We chose this design with the microdose in addition to the same therapeutic drug because it was one of the first microdosing studies in children, and it was likely more acceptable to the ethics board and parents. We are now convinced that this is not a major issue for parents, who understood the concept of microdosing clearly and realized it was associated with minimal burden for the patient. Another limitation is the relatively low number of neonates meeting inclusion criteria. Metabolites other than AAP-glu and AAP-sul were not measured, and no conclusion can be made on the oxidation of AAP. Finally, renal function was not taken into account in the analyses using urinary ratios of the parent compound and metabolite (U-AAP-glu/U-AAP and U-AAP-sul/U-AAP). These ratios are indirect markers and might also reflect renal developmental changes; therefore, our results may have underestimated the developmental change, as has been shown for urinary markers for cytochrome P450 2D6 [44].

## 5 Conclusion

We show the feasibility of using a [ $^{14}\text{C}$ ]-drug microdose to phenotype the ontogeny of drug metabolism in children. The previously reported developmental change of paracetamol metabolism from mainly sulfation in neonates to glucuronidation in older children was comparable. From this study, plasma metabolite data were added on the combined intestinal and hepatic pathways in a large pediatric age range. The success of this study design is

promising for future studies on the development of drug disposition.

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**Author contributions** Participated in research design: MGM, WHV, SNW, CAJK, ADW, HH, JR, DT. Organized inclusion and sample collection: MGM, SNW. Contributed new reagents or analytic tools: ED, WHV, SNW, AD, HH. Performed data analysis: MGM, JR, SNW, CAJK, DT. Wrote or contributed to the writing of the manuscript: MGM, ED, JR, WHV, KA, CAJK, DT, SNW.

## Compliance with Ethical Standards

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**Conflict of interest** M.G. Mooij, E. van Duijn, C.A.J. Knibbe, K. Allegaert, A.D. Windhorst, J. van Rosmalen, N.H. Hendrikse, D. Tibboel, W.H.J. Vaes, and S.N. de Wildt declare they have no conflicts of interest.

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