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This thesis describes the potential role of non-invasive measurement of pharmacokinetics (PK) and pharmacodynamics (PD) in the research and development of central nervous system (CNS) stimulants or depressants for children and adolescents. First, we evaluated the feasibility of using saliva as an alternative to plasma in two studies on psychostimulants (caffeine and methylphenidate). Second, neuropsychological and neurophysiological functions were measured longitudinally using the NeuroCart, a battery of tests developed at the Centre for Human Drug Research (CHDR, Leiden, The Netherlands) that includes non-invasive tests for alertness, visuomotor coordination, motor control, memory, and subjective drug effects. Using a non-invasive approach, age-dependent differences in alcohol metabolism, drug-related functional activity in the brain is to measure drug-related CNS functional activity using methods that provide sufficient sensitivity and specificity. To relate drug-related changes in CNS functional activity to changes in PK, drug concentrations must be measured. Traditional PK protocols—with multiple samples and indwelling catheters or multiple venipunctures—are undesirable in therapeutic pediatric drug research. To overcome some of these limitations, other sample collection methods for determining drug concentration (for example, saliva sampling) have been developed and validated. Saliva sampling has the added benefit of allowing on-site testing without the need for medical personnel or complicated post-collection sample processing, thereby further decreasing the burden placed on the children. Unfortunately, however, the usefulness of determining the saliva concentration of several drugs has been questioned because of variability in the saliva:plasma concentration (S/P) ratio. We propose that if the sources of variability in the S/P ratio can be quantified or minimized, measuring the saliva drug concentration might be a meaningful alternative to measuring plasma drug concentration.

**CHAPTER 1: INTRODUCTION**

The pharmacological treatment of children and adolescents with diseases of the CNS has traditionally followed the development program in adults. However, because of differences in neuropsychopathology and development-specific differences in PK and/or PD, the relationships between drug action and drug exposure in children cannot be understood fully by simply extrapolating information from adult patients. Therefore, it is important to investigate age-dependent differences in PK and PD. Unfortunately, our understanding of these differences is based almost entirely on animal models. In addition, the registration of CNS drugs for use in children and adolescents has lagged behind new developments in adults. The situation has been complicated even further by a decrease in the number of new drug registrations for psychiatric and neurological indications in adults.

Despite these factors, the number of treated children and adolescents, as well as the duration of exposure to CNS drugs, has increased substantially over the past few decades. Recent European legislation (the EU Pediatric Regulation) will likely drive an increase in pediatric trials and specific label changes, dosing recommendations, and age-appropriate formulations.

Several challenges have emerged when working within the framework of this new legislation and there is an urgent need for validated assessment tools that are suitable for evaluating the efficacy and safety of CNS drugs in the pediatric population. In addition, researchers should attempt to reduce the burden placed on participating children and adolescents by using non-invasive or minimally invasive measurement methods.

The most accessible and non-invasive means to measure drug activity in the brain is to measure drug-related CNS functional activity using methods that provide sufficient sensitivity and specificity. To relate drug-related changes in CNS functional activity to changes in PK, drug concentrations must be measured. Traditional PK protocols—with multiple samples and indwelling catheters or multiple venipunctures—are undesirable in therapeutic pediatric drug research. To overcome some of these limitations, other sample collection methods for determining drug concentration (for example, saliva sampling) have been developed and validated. Saliva sampling has the added benefit of allowing on-site testing without the need for medical personnel or complicated post-collection sample processing, thereby further decreasing the burden placed on the children. Unfortunately, however, the usefulness of determining the saliva concentration of several drugs has been questioned because of variability in the saliva:plasma concentration (S/P) ratio. We propose that if the sources of variability in the S/P ratio can be quantified or minimized, measuring the saliva drug concentration might be a meaningful alternative to measuring plasma drug concentration.

**CHAPTER 2: THE EUROPEAN PEDIATRIC REGULATION: WILL IT PROVIDE CHILDREN WITH THE MEDICINES THEY NEED?**

In this chapter, a study is described in which the impact of the European Pediatric Regulation on the development of pediatric medicines—including CNS drugs—is evaluated. The Regulation requires the pharmaceutical industry to plan clinical trials in children in an early stage during drug development in adults or in case a new indication, formulation or administration route is investigated for adults for on-patent medicines. The so-called Pediatric
In children, this plan should be presented by the company early in the development of a medicine and subsequently agreed with the Pediatric Committee of the EMA. In this study, we evaluated the drug classes for which pediatric development was either agreed for development or was waived by the EMA from 2007 until March 2012. In addition, we evaluated whether the Regulation is likely to lead to the development of drug classes for which there exists a (unmet) pediatric need, or for which pharmaceutical expenditure is high. In addition, Dutch physicians working in pediatric healthcare indicated if they find that the pediatric medicines (developed and researched under the Regulation) are actually needed.

From 2007 until March 2012, approximately two-thirds of the medicines were agreed by the EMA for pediatric development; deferral of the start or completion of measures in the Pip until after authorization for adults was granted for 83% of these medicines. Drug classes and therapeutic subgroups with a high need for pediatric research and development on the EMA Needs Lists, like CNS drugs, are either researched infrequently or often waived from pediatric development. In addition, medicines that are frequently prescribed (PhARMO Database Network, 2005-2011), but are not always readily available (and that therefore represent an unmet pediatric need) are researched relatively rarely under the Regulation. The drug classes in our evaluation with the lowest number of medicines with agreed pediatric development had the lowest pharmaceutical expenditure (GIP databank, 2007-2011). Finally, fifty Dutch physicians working in pediatric healthcare were not convinced that medicines for which pediatric development was agreed are needed for their clinical practice. Our analysis confirms the increased need for researching and developing CNS drugs for use in pediatric patients, as drugs acting on the nervous system (the so-called neurologicals) had the highest number of off-label active substances for which the EMA Needs Lists identified a need. Nearly half of all neurologicals listed were indicated to need full pediatric development. Anesthetics, analgesics, antiepileptics, and psycholeptics (such as antipsychotics, anxiolytics, hypnotics, and sedatives) were among the therapeutic subgroups with the highest number of medicines with a pediatric need. Our analysis also indicates that the percentage and number of children and adolescents who are treated using CNS drugs continues to rise, reflected by an annual increase in outpatient use of neurologicals in Dutch children and adolescents from 2005 through 2011 (the end of the study period).

We conclude that the Regulation’s key strategy does not necessarily lead to the increased pediatric development of drug classes for which there may be a (unmet) pediatric need. Instead, the Regulation’s output is in line with expenditure data, most likely as a result of the ‘adult-driven’ approach. In addition, given the high number of granted deferrals it is likely that the registration of CNS drugs for use in children and adolescents still lags behind new developments in adults. We propose that important refinements in implementation are needed in order to ensure that the Regulation will provide children and adolescents with the medicines they actually need. For example, evaluation of any proposal for a Pip (or request for a waiver) should be based on the potential pediatric relevance of the mechanism of action or drug target, and new incentives should be considered for first-in-child indications.

CHAPTER 3: BIOMARKERS OF ACUTE METHYLPHENIDATE EFFECTS IN CHILDREN AND ADOLESCENTS WITH ATTENTION-DEFICIT/HYPERACTIVITY DISORDER

The psychostimulant methylphenidate (MPH) is the most commonly prescribed medication for treating pediatric attention-deficit/hyperactivity disorder (ADHD). Previously published studies investigating the effects of immediate-release MPH (MPH-IR) yielded contradictory results due to several sources of variability, for example a lack of standardized biomarkers for drug-effect measurements.

In the study described in chapter 3, we performed a systematic literature review to identify sensitive and useful non-invasive biomarkers for monitoring the effects of MPH-IR in children and adolescents with ADHD. We identified 78 randomized placebo-controlled clinical studies (published until December 2009) that investigated CNS effects following a single dose of MPH-IR in pediatric ADHD patients. Outcome measures were clustered to groups of related
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**Summary**

In adults, clinical monitoring of MPH therapy is usually performed by measuring plasma MPH concentrations. In children, however, blood sampling is undesirable. Saliva may be an alternative matrix for monitoring MPH concentrations; however, several potential complicating factors have been encountered in previous studies. Complicating factors included indications of oral contamination in the first few saliva samples after taking MPH tablets and considerable—yet unexplained—variation in the s/p ratio throughout the time course of both tablet and capsule formulations.

Obtaining an accurate s/p ratio is essential for realizing the full potential of using saliva sampling to monitor plasma MPH concentrations. Therefore, we developed an analytical method for accurate and precise quantification of MPH in both plasma and saliva. In this chapter, we present the validation of a liquid chromatography–tandem mass spectrometric method using a hydrophilic interaction liquid chromatography column (HILIC). In a 100 µl sample, proteins were precipitated with 750 µl acetonitrile/methanol 84/16 (v/v) containing d9-methylphenidate as the internal standard. Standard curves were prepared over the MPH concentration range of 0.5 – 100.0 µg/l. The total analysis time was 45 seconds. Accuracy and within- and between-run imprecision were in the range of 98-108% and less than 7.0%, respectively. Matrix effects were greater for plasma than saliva with 46% and 8% ionization suppression. The matrix effects were adequately compensated by the use of deuterated MPH as internal standard. MPH significantly degraded in plasma and saliva at room temperature and 5°C. Stability experiments demonstrated that samples should be stored at temperatures of -20°C or below directly after sampling, and that samples should be processed immediately after thawing. Samples were stable at -20°C for at least 4 weeks. The method was successfully applied for the determination of MPH concentrations in plasma and saliva samples from an adult healthy volunteer.

We conclude that using protein precipitation and hydrophilic interaction liquid chromatography coupled to tandem mass spectrometry, this method allows fast, accurate and precise quantification of MPH in both plasma and saliva.
In this chapter, a study is described in which a first attempt was made to quantify sources of variability in MPH plasma and saliva concentrations, and to describe the relationship between MPH concentration in saliva and MPH concentration in plasma using a population PK modeling approach.

In this randomized, open-label study, MPH-IR (tablet) and osmotic release oral system MPH (MPH-OROS, capsule) were administered in a crossover design to 12 healthy adult subjects (six men and six women). Paired blood and saliva samples were collected pre-dose and at regular intervals for 6 (MPH-IR) or 11 (MPH-OROS) hours following drug administration. Population PK analysis was performed using nonlinear mixed-effect modeling.

A one-compartmental structure model with first-order absorption (with separate compartments for MPH-IR and MPH-OROS) and first-order elimination provided the best description of estimated MPH plasma PK. The estimated clearance was 6.0 liters/hour and the volume of distribution was 7.5 liters. The derived terminal half-life was 0.9 hours. Inter-individual variability was identified on clearance, the volume of distribution, and the absorption rate constant for MPH-OROS. The S/P ratio was 2.44 from 2.5 hours onward. Inter-individual variability was identified in the S/P ratio.

With proper allometric scaling techniques, we expect that this PK model can be used in children to predict the concentration-time profile in the plasma using MPH concentrations measured in saliva samples. Further studies are needed to determine the predictive performance of the model in children with ADHD.
that described saliva caffeine concentrations in adults >1 hour after administration as a fraction (0.68) of plasma concentration (i.e., the s/p ratio was 0.68). Before 1 hour after administration, saliva caffeine concentrations could not be described as a linear fraction; therefore, caffeine’s early effects in adolescents were not suitable for inclusion in a PK/PD model.

We conclude that in healthy, alert adolescents, 90 mg caffeine has significant effects on parameters regarding alertness and reaction speed, despite the relatively low dose and the expected ceiling effect in this healthy and alert population. Whether these effects observed in adolescents are larger in adolescents than in adults remains to be determined.

CHAPTER 7: COMPARISON OF THE PHARMACOKINETICS AND EFFECTS OF ALCOHOL ON OBJECTIVE AND SUBJECTIVE BIOMARKERS BETWEEN HEALTHY ADOLESCENTS AND ADULTS

Our understanding of age-dependent differences in PK and PD of CNS drugs is based almost entirely on animal models. In this study, we used a PK/PD modeling approach to compare the objective and subjective responses to alcohol between adolescent and adult subjects. The acute effect of consuming a socially accepted dose of alcohol (two standard units) was determined in 16-18-year-old adolescents. Blood alcohol concentration was measured non-invasively using end-expired breath samples. A PK/PD model was then developed by combining the data obtained from this study in adolescents with data obtained from previous alcohol studies (using the clamping method) performed in adults. This model was used to characterize alcohol’s PK and effects on an objective biomarker and a subjective biomarker and to explore potential sources of variability, including age.

A two-compartment structural model with first-order absorption and Michaelis-Menten elimination provided the best description of estimated plasma alcohol PK. Inter-individual variability was identified for several kinetics parameters, with lean body weight-dependent variability in peripheral compartment volume and maximum elimination, weight-dependent variability in central compartment volume, and height-dependent and age-dependent variability in intercompartment clearance.

Smooth pursuit performance and vas Alertness were selected as biomarkers for PK/PD modelling based on an exploratory meta-analysis of all relevant alcohol data. The relationship between alcohol concentration and the effects of alcohol on baseline smooth pursuit performance and vas Alertness score was described best as being dose-dependent, with no indications of delay or tolerance. Higher baseline performance for smooth pursuit was correlated with a larger absolute decrease in performance. No covariates were identified for the relationship between alcohol concentration and effect with respect to smooth pursuit performance or vas Alertness score.

The covariates that were identified in our PK model may be (in)directly related to differences between adolescents and adults, as considerable age-related and maturity-related changes in body composition occur during adolescence. vas Alertness and smooth pursuit eye movements may not necessarily represent all of the alcohol-related effects on the CNS, and we cannot exclude the possibility that sensitivity to other alcohol-related pharmacodynamics effects change with age. Unfortunately, other sensitive functional biomarkers for alcohol effects were less suitable for developing a PK/PD model, as the small effect in adolescents and the high number of non-responders precluded our ability to quantify the adolescent data and evaluate an age-dependent effect. Therefore, whether sensitivity to other alcohol-related pharmacodynamics effects changes with age remains to be determined.

CHAPTER 8: PHARMACOKINETICS OF PROLONGED-RELEASE MELATONIN MINI-TABLETS IN CHILDREN WITH BOTH AUTISM SPECTRUM DISORDER AND A SLEEP DISORDER

Melatonin is one of the CNS drugs identified by the EMA as having a pediatric therapeutic need. This includes the need to develop an age-appropriate sustained-release formulation and the need to collect data regarding melatonin’s pharmacokinetics.
PK, efficacy, and safety in children with autism spectrum disorder (ASD) and a sleep disorder. The study described in chapter 8 is part of a Pediatric Investigation Plan (PIP) under the Pediatric Regulation. This was a cross-over ascending dose study of Circadin (1 mg 3-mm diameter mini-tablets), a prolonged-release melatonin formulation. In this study, the PK profile, safety, and acceptability of Circadin were evaluated in 16 children and adolescents with autism and a sleep disorder. We tested 2 mg and 10 mg doses of Circadin based on the dose range we will use in an upcoming efficacy trial, which is also part of the development plan. To minimize the number of samples taken during the night, Circadin was administered in the early morning. The first occasion included a 24-hour baseline measurement day. Whole-saliva samples were collected non-invasively from passive drool, and melatonin concentration was measured. Urine samples were collected for determination of the metabolite 6-sulphatoxymelatonin (6-SMT). Adverse events were monitored throughout the study, and sedative effects were assessed using the Observer’s Assessment of Alertness/Sedation (OAA/S) scale for 10 hours after administration. PK parameters for melatonin were estimated using non-compartmental modeling.

All 16 subjects (12 male, 4 female; age range: 7-15 years) had a clinical diagnosis of autism spectrum disorder (based on DSM-IV-TR criteria). All reported side effects were consistent with known side effects. Mini-tablets were found to be both safe (i.e., none of the children choked) and acceptable to the children. The melatonin concentration peaked within two hours of administration and remained elevated for several hours thereafter. Circadin exposure was dose-linear, and clearance (1,000 l/hr) was similar between the dose groups. The median apparent terminal half-life was comparable between dosages. The mean total 6-SMT recovered from urine during the baseline period was 4.2 µg/12 daytime hours and 13.5 µg/12 nighttime hours. Following administration with Circadin 2 mg, the mean amount of total 6-SMT recovered from urine was 98.9 µg/12 daytime hours and 95.3 µg/12 nighttime hours. The highest levels of sedation (assessed using the OAA/S) were observed between 2 and 3 hours after administration of Circadin 2 mg and between 2 and 6 hours after administration of Circadin 10 mg. Overall, the subjects and their caregivers were positive about the burden and duration of the study. Nearly all subjects (69%) and caregivers (88%) stated that they would consider (consent for) participating in a similar trial again.

We conclude that this study demonstrates the short-term safety, acceptability, and prolonged-release profile of Circadin mini-tablets in 16 school-age children and adolescents with ASD.

Chapter 9: Pharmacokinetics and Pharmacodynamics of a New Highly Concentrated Intranasal Midazolam Formulation for Conscious Sedation

Because of its rapid onset and rapid recovery profile, midazolam is the medication of choice for providing conscious sedation and management of epileptic seizures. Nasal delivery of midazolam is a non-invasive alternative to intravenous administration. However, previous formulations for delivering midazolam nasally have not been very successful due to the lack of solvents that can dissolve midazolam at therapeutic dosages without causing nasal mucosa damage.

In the study described in chapter 9, the pharmacokinetics of two doses of a novel highly concentrated aqueous intranasal midazolam formulation (Nazolam) was characterized. In this four-way crossover, double-blind, double-dummy, randomized, placebo-controlled study, 16 subjects received 2.5 mg Nazolam, 5.0 mg Nazolam, 2.5 mg intravenous midazolam or placebo on different occasions. Pharmacokinetics of midazolam and 6-hydroxy-midazolam were characterized and related to outcome variables for sedation (saccadic peak velocity, the Bond and Lader Visual Analogue Scale for sedation, the simple reaction time task and the Observer’s Assessment of Alertness/Sedation). The onset and duration of the pharmacological effect were evaluated and compared to intravenous midazolam using the biomarker saccadic peak velocity (SPV), as a relationship between SPV reduction and clinical efficacy has been described. Nasal tolerance was evaluated through subject reporting and ENT examination.
Bio-availability of Nazolam was 75%. Maximal plasma concentrations of 31 ng/ml (cv, 42.3%) were reached after 11 minutes (2.5 mg Nazolam), and of 66 ng/ml (cv, 31.5%) after 14 minutes (5.0 mg Nazolam). Sedation onset (based on -250 spv change) occurred 1 minute after administration of 2.5 mg intravenous midazolam, 7 minutes after 2.5 mg Nazolam, and 4 minutes after 5 mg Nazolam. Sedation duration was 85 minutes for 2.5 mg intravenous midazolam, 47 minutes for 2.5 mg Nazolam, and 106 minutes for 5.0 mg Nazolam. Clinically relevant levels of sedation (as measured using OAA/S) were achieved within minutes after administration. In addition, single administration was well tolerated and safe, and did not lead to nasal mucosa damage.

We conclude that this study demonstrates the nasal tolerance, short-term safety and efficacy of this novel formulation in 16 healthy adult subjects. When considering the preparation time needed for obtaining venous access, conscious sedation can be achieved in the same time span as needed for intravenous midazolam. This non-invasive formulation may offer important advantages in conscious sedation and epilepsy and has potential for use in children.

Chapter 10: General Discussion

CNS drugs are researched infrequently under the Regulation, despite the high need for pediatric research and development, and despite the ongoing increase in the use of these medicines among pediatric patients. Based on our data, it is unlikely that the Regulation will have a positive impact on the ability of children and adolescents to access new CNS drugs (as only few new CNS drugs are developed for adults), or on the delay in pediatric registration of CNS drugs as described in the Introduction to this thesis. Given the high number of CNS drugs with deferred pediatric studies, clinical trial strategies should be revised in a timely fashion.

In the introduction to this thesis, we proposed that if variability in the S/P ratio can be minimized or quantified, measuring the saliva drug concentration might be a meaningful alternative (to measuring plasma drug concentration) for more drug types than has seemed feasible until now. The studies described in chapters 5 and 6 attempted to investigate and quantify the sources of this variability using a population PK modeling approach. We showed that the relationship between plasma and saliva concentrations of MPH is stable beyond 2.5 hours and of caffeine beyond one hour after administration. Prior to these time points, the relationship between plasma and saliva concentrations was not linear; unfortunately, our efforts to correct for factors that potentially contribute to variability in the S/P ratio were unsuccessful. The number of saliva samples that can be collected to measure the absorption phase of compounds with a short Tmax (such as MPH and caffeine) is limited, thus hampering the ability to determine the magnitude of contamination, the S/P ratio, and sources of variability in the S/P ratio directly following administration. For these drug types, only CNS effects that occur relatively late are suitable for developing a PK/PD model using measured saliva concentrations or predicted plasma concentrations. However, because the pharmacological CNS action of most compounds is delayed relative to changes in plasma concentration, saliva sampling may still be a viable option for this type of research. Therefore, our results support the further exploration of using saliva as a non-invasive method for drug profiling CNS drugs.

The non-invasive CNS tests that are included in the NeuroCart battery are sensitive enough to detect effects of low doses of caffeine and alcohol in healthy adolescents, as shown by the studies described in chapters 6 and 7. Significant effects on parameters regarding alertness (saccadic peak velocity) and reaction time (adaptive tracking) were observed after caffeine was administered, despite the relatively low dose and the expected ceiling effect on several parameters in this cohort of healthy, alert adolescents. Because caffeine has been reported to produce behavioral effects (including motor activation and arousal) similar to the effect of classic psychostimulants such as cocaine and amphetamine, we proposed that these tasks could be valuable in pediatric studies using other CNS stimulants. In the study described in chapter 7, a low dose of alcohol induced significant changes in smooth pursuit eye movements, vas alertness score, the vas alcohol effect score, body sway, systolic blood pressure, and heart rate. Animal studies have revealed...
developmental changes in the pharmacological sensitivity of GABA<sub>A</sub>-receptor-mediated currents to several drugs. Therefore, these tasks could be used to study age-related differences in the CNS effects of GABA-ergic compounds in pediatric drug research. Importantly, the CNS tests and their duration were tolerated by the majority of adolescent subjects in these clinical studies.

In the introduction to this thesis, we raised several issues with respect to pediatric neuropsychopharmacology. First, the differences in neuropsychopharmacology and pharmacology between children and adults must be recognized. In addition, researchers need validated tools that are appropriate for assessing the efficacy and safety of CNS drugs in children. Finally, special emphasis should be placed on formulation research. We propose that CNS drug profiling has the potential to address all of these issues. First, CNS drug profiling enables researchers to evaluate age-dependent effects by drawing comparisons with existing data collected in adults, as demonstrated by the study described in chapter 7. Second, several drug classes have a unique CNS drug profile on the NeuroCart test battery, which corresponds to the drug class’ mechanism of action. We have demonstrated previously that these ‘fingerprints’ can be used to differentiate a drug’s stimulant and sedative properties, for example by evaluating effects on saccadic peak velocity. As mentioned already, the CNS tests that are included in the NeuroCart battery are sensitive enough to detect low doses of caffeine and alcohol in adolescents. Functional biomarkers could be used to determine whether a specific drug exerts a pharmacological effect at a specific dose in children, and they could also be used to monitor unwanted sedative or stimulant properties of drugs in children. Third, non-invasive monitoring of PK and PD could facilitate the evaluation of age-appropriate formulations of CNS drugs for use in children, as illustrated by the clinical studies described in chapters 8 and 9. The ‘proof-of-pharmacology’ strategy described in chapter 9 may perhaps also be followed in pediatric studies on drugs for which extensive data exist on efficacy and safety; but for which there is only a need for an age-appropriate formulation.

In parallel with further refining the methods described in this thesis, several other steps must be taken. First, non-invasive drug monitoring should be evaluated in younger age groups and in subjects with other neuropsychiatric disorders. A second important step, particularly considering that most neurological and psychiatric disorders are either chronic or recurrent, is to determine whether early treatment translates into an acute improvement in symptom-related CNS functions as well as improved long-term outcome. Finally, as there is a need to study the effects of anesthetics and analgesics in children and adolescents, more experience should be gained in the field of pediatric pain research.

**CONCLUSION**

The clinical studies included in this thesis show that non-invasive drug profiling for CNS stimulants or depressants is feasible in healthy adolescents and children with ASD. In addition, non-invasive drug profiling may help researchers both evaluate age-dependent PK and PD and compare the effect profiles of various formulations. Importantly, this approach may also facilitate the execution of studies included in Pediatric Investigation Plans under the Pediatric Regulation, thus representing an important step forward, particularly given the high need for pediatric research with respect to CNS drugs. The ideal pediatric study should be executable in the subjects’ homes. Adult studies of novel drugs that could potentially be measured in saliva samples should include measurements of the S/P concentration ratio. Because considerable PK variability has been reported for several neuropsychiatric drugs, sensitive non-invasive or minimally invasive PD measurements should be assessed longitudinally. In addition, pediatric studies should ideally include a means to compare the results with adult studies. Finally, to expand our knowledge regarding the acceptability and tolerability of measurement methods, whenever possible the study participants should be asked to complete a questionnaire in order to collect information regarding the reasons why children and adolescents participate, as well as their perceived burden associated with the study.