# CHAPTER 10

## General discussion

## *CNS drug research under the Pediatric Regulation: how can we move forward?*

The 2007 EU Pediatric Regulation requires the industry to plan clinical trials in children early in the development of new drugs for use in adults, or for line extensions for on-patent drugs (unless a waiver or deferral has been granted). Because the Regulation is at the core of pediatric needs and establishes clear obligations and a system of incentives aimed at the pharmaceutical industry, the Regulation was expected to result in research that would be focused more on the needs of children and would therefore drive important changes in therapeutic options available to pediatric patients[1]. Based on our evaluation presented in chapter 2, it is clear that under the Regulation, a higher percentage of medicines are considered for use in children. However, the Regulation does not necessarily lead to the increased pediatric development of drug classes for which there may be a unmet pediatric need based on pediatric usage and availability data. Importantly, only seven therapeutic subgroups accounted for half of all medicines for which pediatric development was agreed, and the drug classes that the European Medicines Agency (EMA) Needs Lists identified as needing pediatric research and development were researched relatively infrequently under the Regulation. In addition, a small contingent of Dutch physicians working in pediatric healthcare are not convinced that medicines for which pediatric development was agreed are needed for clinical practice.

Our analysis confirms the increased need for researching and developing central nervous system (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 medicines for which the EMA Needs Lists identified a need[2]. Nearly half of all CNS drugs 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 active substances 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 out-patient use of neurologicals in Dutch children and adolescents from 2005 through 2011 (the end of the study period). The largest increase was among antidepressants (including antidepressants combined with psycholeptics) and stimulants. The in-patient use of CNS drugs was not evaluated, but is also likely substantial, given that neonatologists, pediatricians, pediatric neurologists, and child and adolescent psychiatrists commonly prescribe neuropsychiatric drugs.

Despite this high need for pediatric research and development, and despite the ongoing increase in the use of CNS drugs among pediatric patients, CNS drugs are researched only rarely under the Regulation. In addition, the Regulation is not likely to have a positive impact on the ability of children and adolescents to access new CNS drugs, or on the delay in pediatric registration of CNS drugs as described in the Introduction to this thesis. A total of 32 CNS drugs were agreed for pediatric development, the majority of which (approximately 80%) had a unique Anatomical Therapeutic Chemical (ATC) code in the WHOCC (World Health Organization Collaborating Center) database (not reported in chapter 2), indicating that these drugs are currently marketed in at least one country. The newly developed CNS drugs included several novel drugs; however, the development of some of these drugs had already been discontinued[3]. In addition, for the majority (81%) of CNS drugs with agreed pediatric development, one or more pediatric development plans included at least one measure for which deferral was granted until the drug was authorized for marketing to adults. The relatively low number of CNS drugs with agreed pediatric development is likely due to the reported decrease in research in adults. Because the adult indication is the starting point of the Regulation, and because pediatric development plans are submitted as part of a development plan in adults, drug development for children with neuropsychiatric disorders still follows adult development, although some pediatric epilepsy syndromes (such as neonatal seizures, Lennox-Gastaut syndrome and Dravet syndrome) are being addressed specifically under the Regulation.

To address some of these issues, in chapter 2 we suggested that the evaluation of any proposal for a pediatric investigation plan (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-children indications. In addition, given the high number of CNS drugs with deferred pediatric studies, clinical trial strategies should be revised in a timely fashion. Deferrals under the Pediatric Regulation may be related in part to the need for extra time due to a lack of general expertise, as pediatric research in the EU is less extensive than in the United States. For example, in the field of child and adolescent psychopharmacology, the majority of publications and studies originate in the US[4], and the ability to run early drug trials with innovative therapies is significantly higher in the US than in Europe[5]. Because clinical research in pediatric patients is hampered by interrelated logistic and ethical constraints – including a limited number, extent, and invasiveness of study-related interventions that can be performed if they are not part of routine clinical care – researchers should attempt to reduce the burden placed on participating children and adolescents by using non-invasive or minimally invasive measurement methods. Changes in methods that are designed to reduce the patient's burden (for example, changing the sampling procedure) have already been reported to increase patient enrollment in studies of rare pediatric diseases that were performed under the Regulation[6]. Therefore, in this thesis, we explored the feasibility of using non-invasive monitoring of pharmacokinetics (PK) and pharmacodynamics (PD) for pharmacological drug profiling of commonly used CNS stimulants and depressants in children and/or adolescents. Drug concentrations were measured non-invasively in either the saliva or exhaled breath, and neurocognitive and neurophysiological functions were measured longitudinally using the NeuroCart test battery.

This chapter reviews how this approach was used in the preceding chapters of this thesis. We will first discuss the feasibility and applicability of saliva sampling in pediatric populations based on data obtained from pediatric clinical studies of caffeine, methylphenidate, and melatonin. Then, we will discuss the (potential of) pharmacological profiling of pharmacodynamics in children and adolescents based on literature review and clinical studies of methylphenidate, caffeine, alcohol and midazolam. This chapter concludes with potential practical applications of this approach and suggestions for future directions.



## *Non-invasive drug profiling in children and adolescents*

### PHARMACOKINETICS

In chapters 6 and 8, we evaluated the feasibility and applicability of sampling saliva from healthy adolescents and from children and adolescents with autism spectrum disorder (ASD). Although the sample collection devices and techniques (for example active versus passive collection) are dictated by the characteristics of the drug of interest, the collection method must be tolerated by children and adolescents. As we expected, active sampling using a Salivette collection device was tolerated well by the adolescents who participated in the caffeine study (chapter 6). Children with ASD can have particularly sensitive sensory systems[7] that can cause the child to resist certain collection devices. In the study described in chapter 8, we collected whole saliva samples from children and adolescents with ASD; whole saliva sampling is the preferred method for measuring melatonin concentrations in saliva[8]. A recent study of 6-12-year-old (mostly male) children with ASD found that passive saliva sampling was an acceptable collection method (in terms of ease and comfort)[9], and this method can also be used in young children[10] without associated risks such as choking. In our study, we used the Saliva Collection Aid (available from Salimetrics Europe), a device that was recently developed to simplify the collection of whole saliva from passive drool. Importantly, the device is constructed of polypropylene, which resists sample retention and contamination, issues that can occur with amines such as melatonin. This collection method was tolerated well by the subjects in our study.

Due to difficulties in reliably predicting plasma concentration using saliva measurements, determining the concentration of a drug in a saliva sample was limited for basic drugs (which undergo an alkaline reaction in aqueous solutions) and for acidic drugs and drugs that are highly protein-bound (these drugs can have an extremely low S/P ratio). However, if sources of variability in the S/P ratio can be overcome for these drug types, measuring saliva drug concentration might be a feasible alternative to measuring plasma drug concentration. The studies described in chapters 5 and 6 attempted to investigate and quantify the sources of variability (for example, contamination and

saliva pH) in the plasma and saliva MPH and caffeine concentrations measured using a population PK modeling approach. In previous studies, the S/P ratios of MPH[11] and caffeine[12–14] concentrations were time-dependent, possibly due to fluctuations in arteriovenous blood concentration[12–14] and/or pH partitioning[11,13]. In addition, because many drugs are administered orally, contamination in the saliva can influence the S/P ratio at early time points after administration. In chapters 5 and 6, we showed that the relationships between plasma and saliva concentrations of both MPH and caffeine are stable (i.e., not time-dependent or concentration-dependent) after 2.5 hours (for MPH) and one hour (for caffeine) of administration. Prior to these time points, the relationship between plasma and saliva concentrations was not linear.

Sample contamination due to residual drug levels is a general concern in pediatric clinical research, but this issue is rarely addressed or taken into account. For example, in oncology research, PK measurements are usually taken from samples obtained directly from the indwelling central venous line (CVL), which is the same line through which intravenous chemotherapeutics are administered. This is usually due to practical considerations (as the line is already in place) and prevents the need to collect samples from a peripheral catheter (which would need to be inserted), thereby increasing study enrollment. Although studies suggest that clearing the line can minimize the contamination of certain drugs, there is currently no universally accepted method to reliably address this issue. One exception is a recently published paper by Edwards and colleagues[15], who developed a population PK model of actinomycin-D in children with cancer by incorporating expressions that account for drug contamination from samples obtained via an indwelling CVL. Compared to other models, their baseline contamination model – including a contamination factor proportional to the model-predicted concentration for samples obtained from a CVL – was chosen as the most conservative and accurate model. This contamination model assumed that drug contamination from the sampling catheter was included in the baseline concentration and was therefore factored into the level of each individual prediction. Because many CNS drugs are delivered orally, sample contamination can also play a role when using saliva sampling. For example, remnants of syrup, uncoated tablets, or chewable tablets can contaminate saliva samples and can cause inappropriately high drug concentrations in samples measured early after administration. This source of contamination can be largely eliminated by thoroughly rinsing the mouth both after taking the drug and prior to obtaining a saliva sample[16], as was done in the studies described in this thesis. However, even if the mouth is rinsed thoroughly, saliva concentrations can be higher than expected due to residual contamination, transient transmucosal absorption or deposition of the drug. Therefore, in the studies described in chapters 5 and 6, we attempted to compare the magnitude of contamination after drinking a beverage (caffeine) or swallowing a tablet (an immediate-release MPH tablet) with contamination after swallowing a sealed capsule. Using this approach, we anticipated that a contamination factor (the elimination rate constant) could be incorporated into the model equation describing the saliva PK data, and model-predicted saliva concentrations (and hence the S/P ratio) could be individually corrected for the level of contamination. In addition, the half-life of the elimination rate constant could be used to determine the time window in which considerable contamination could be expected, thus providing information regarding sampling time points for therapeutic drug monitoring in clinical practice. Unfortunately, our efforts to correct for oral contamination were largely unsuccessful, due in part to the relatively few observations measured at early time points following drug administration; therefore, this approach was abandoned.

Even in cases in which the saliva sample is not contaminated, the S/P ratio may not be consistent across PK phases. Several factors may account for this inconsistency[17]. First, the arteriovenous concentration ratio can vary between PK phases, particularly for compounds that diffuse easily. Second, even in the absence of this phenomenon, the S/P ratio may not be stable throughout all PK phases. If elimination is linear and proceeds at the same rate in plasma and saliva (i.e., parallel decline), the ratio will be inconsistent; however, if elimination proceeds exponentially, the ratio will remain constant, with a parallel decline in plasma and saliva drug concentrations. Third, concentration-dependent

protein binding can account for differences in the S/P ratio within an individual subject. Finally, pH partitioning can play a role, particularly for basic drugs, which undergo an alkaline reaction in aqueous solutions. Many CNS drugs are basic and reside in equilibrium between their charged and neutral states under physiological conditions. Thus, the free fraction of the ionized drug can be incorporated in saliva, as saliva is slightly more acidic than plasma[18]. Therefore, the S/P ratio is highly sensitive to small changes in saliva pH, which in turn can be influenced by saliva flow. In the studies described in chapters 5 and 6, we measured saliva pH and saliva flow at each saliva sampling time point in order to determine whether changes in these parameters (despite active sampling) accounted for some of the residual variability in the S/P ratio. However, no significant covariates were identified for the S/P ratio.

The feasibility of using non-invasive saliva samples to measure PK is limited by the period of time needed by the subject between sampling time points to replace the saliva sampled. Thus, the number of samples that can be collected to measure the absorption phase of compounds with a short $T_{max}$ (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, feasible, and acceptable method for drug profiling CNS drugs. Given the potential influence of saliva contamination and saliva pH on measured saliva drug concentration, standardizing the collection method (for example, by rinsing the mouth, choosing active versus passive sampling, etc.) will likely improve the applicability of using saliva for drug monitoring. On the other hand, the method is potentially limited by the fact that the relatively low volume of saliva in a sample necessitates the use of a highly sensitive bioanalytical method such as liquid chromatography-tandem mass spectrometry (LC-MS/MS), which can be costly and is currently not generally available for most CNS drugs.

### PHARMACODYNAMICS

The most accessible non-invasive method for assessing the effect of a CNS drug is to measure drug-related CNS functional activity with sufficient sensitivity and specificity[19]. In general, an excessive number of CNS tests are currently used in psychopharmacological research to determine the effects of drugs that act on the nervous system. The sensitivity of these tests for assessing CNS effects has not been determined fully for most drugs, and the reproducibility of these tests may be relatively low. Therefore, careful selection of PD parameters is essential. In the studies described in this thesis, useful CNS tests (or functional biomarkers) to measure the effects of several compounds (including alcohol[20], benzodiazepines[21], and others) in healthy subjects were selected based on previously published studies. These parameters were used to measure PD for alcohol and midazolam in studies of healthy volunteers; these studies are described in chapters 7 and 9, respectively.

Selecting functional biomarkers for measuring drug effects in patients with neuropsychiatric disorders is likely to be more complicated than in healthy volunteers. A useful functional biomarker should be sensitive enough to detect a therapeutic drug dose; in addition, a plausible relationship between the biomarker, drug pharmacology, and/or disease pathophysiology should also be evident[22-26]. Because the precise mechanism of action of many CNS drugs is not fully understood, and because many neuropsychiatric disorders are heterogeneous, these two criteria may be difficult to achieve. The review reported in chapter 3 describes a systematic literature search that used the same approach as previously published reviews in healthy subjects. The aim of our review was to assess the sensitivity and usefulness of functional biomarkers for demonstrating acute CNS effects of immediate-release methylphenidate (MPH-IR) in children and adolescents with attention-deficit/hyperactivity disorder (ADHD). Pediatric ADHD is an exception in the field of pediatric neuropsychopharmacology, as an extensive body of research has been performed

to study the effects of MPH-IR (and other drugs) in this disorder. However, previously published studies investigating the effects of MPH-IR yielded contradictory results due to several sources of variability, including a lack of standardized biomarkers and/or effect measures of MPH[27,28]. The results of our review revealed that a wide variety of biomarkers are currently being used to evaluate the effect of MPH-IR in ADHD. In addition, our review revealed that these studies would benefit greatly from a certain degree of standardization. Because most tests were used relatively rarely, it was difficult to identify the most sensitive tests and drug–response relationships. Nevertheless, despite these limitations, our literature review revealed that the Go/no-go task, the Scale-ADHD, and tests that assess motor control and/or sustained attention may be suitable candidate biomarkers to measure the acute effects of MPH-IR. These tests may facilitate the identification of responders and non-responders following a test dose of MPH-IR. Our evaluation shows that even in the context of extensive pediatric research, it is difficult to reach useful conclusions regarding pharmacological profiles or dose-effect relationships based on currently available studies. For example, several studies in our analysis did not measure drug concentrations, or they performed only a single PD measurement following the test dose. Future studies are needed in order to investigate effect size and to establish clinically relevant changes in CNS test results. Ideally, these studies should include concentration-effect and dose-effect relationships measured at several time points in order to allow for drug effect profiling, similar to the approach used in the clinical studies described in this thesis.

The CNS tests that are included in the NeuroCart battery are sensitive enough to detect low doses of caffeine and alcohol in adolescents, as shown by the studies described in chapters 6 and 7. In the study described in chapter 6, an extensive CNS battery was incorporated in order to obtain information regarding general CNS performance and to identify the functional CNS domains that are affected by caffeine. The CNS tests included saccadic and smooth pursuit eye movements, body sway, adaptive tracking, the left/right distraction task, finger tapping, the attention switch task and the visual and verbal learning task. In addition, blood pressure and heart rate were measured at regular intervals after the administration of caffeine. 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. Improved postural stability and some of the response times approached the level of statistical significance; thus, it is likely that these functions will improve significantly at higher caffeine doses. 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[29], 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, alertness score measured on the visual analog scale (VAS), the VAS alcohol effect score, body sway, systolic blood pressure, and heart rate. Given that animal studies have revealed developmental changes in the pharmacological sensitivity of $GABA_A$-receptor-mediated currents to several drugs (including diazepam[30,31], pentobarbital[30], and zolpidem[32–35]), these tasks could be used to study age-related differences in the CNS effects of GABA-ergic compounds in pediatric drug research. The CNS tests and their duration were tolerated by the majority of adolescent subjects in these clinical studies. These studies demonstrate the feasibility of collecting a rich dataset from this age group, and they show that neuropsychological and psychomotor tasks can be used as biomarkers of the acute effects of low-dose caffeine or alcohol in healthy adolescents.

### *Potential practical applications*

In the introduction (chapter 1) of this thesis, we raised several issues with respect to pediatric neuropsychopharmacology. First, the differences in both neuropsychopathology 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. CNS drug profiling has the potential to address all of these issues, as it enables researchers to evaluate age-dependent differences in PK and PD, and it can facilitate distinguishing between the drug properties of sedatives and stimulants in children. In addition, CNS drug profiling can facilitate comparisons between profiles of drugs with different formulations.

Because our current understanding of age-dependent effects and side-effects is based largely on data collected from animal studies, evaluating age-dependent differences in PK and PD in pediatric clinical trials will be an important step forward. 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. In this study, we used a PK/PD modeling approach to compare the alcohol breath profiles of adolescents and adults, as well as the participants' objective and subjective responses to alcohol. Oral data collected from adolescents were combined in a PK/PD model with previous intravenous data collected from adults. Inter-subject variability could be identified on the basis of various kinetic parameters, and all of the identified covariates were related—either directly or indirectly—to differences between adolescents and adults. Two functional biomarkers were selected based on an exploratory meta-analysis of all PD tasks that were performed several times during a single testing occasion (from several studies). Both smooth pursuit eye performance and VAS alertness responded clearly to alcohol, with no indication of any indirect effects or acute tolerance; thus, it was likely that a relatively simple model would describe the data accurately, and the presence or absence of an age-dependent effect could be investigated. Although we successfully developed a PK/PD model for these functional biomarkers, we did not identify any significant covariates; in particular, we found no clear effect of age. However, VAS alertness and smooth pursuit eye movements may not fully represent every effect of alcohol on the CNS, and we cannot exclude the possibility that sensitivity to other pharmacodynamic effects of alcohol changes with age. Because age-dependent differences may exist for functional biomarkers of acute tolerance (such as subjective intoxication) and/or for tests that evaluate postural stability, it is worth investigating whether sensitivity to other pharmacodynamic effects of alcohol change with age.

Several drug classes—including adenosine antagonists; noradrenergic and serotonergic reuptake inhibitors; $GABA_A$-receptor agonists; ethanol; cannabinoid agonists and antagonists; dopamine antagonists; histamine antagonists; and muscarinic antagonists—have a unique CNS 'fingerprint' (i.e. drug profile) on the NeuroCart test battery. This unique drug profile corresponds to the drug class' mechanism of action[36]. These "fingerprints' can be used to differentiate a drug's stimulant and sedative properties. For example, CNS stimulants can exert a different—or even opposite—effect profile than CNS depressants. CNS stimulants temporarily increase mental and/or physical function and are believed to act primarily on the dopamine, noradrenaline, and serotonin systems or by disinhibiting adenosine. Thus, CNS stimulants may have a common pharmacological profile. By analyzing the results of studies performed previously by our research group and by reviewing other studies (de Mol, unpublished data), we found previously that increased saccadic peak velocity is a typical sign of CNS stimulation. In addition, increased adaptive tracking performance and elevated body temperature were also identified as likely signs of CNS stimulation. Given that benzodiazepines—which are typical CNS sedatives—and ethanol are reported to decrease saccadic peak velocity[23,36], decrease adaptive tracking performance[23], and induce hypothermia[22,37–39], these functional biomarkers might be valuable for providing a better interpretation of the CNS stimulant or sedative effects of drugs that have a modified or novel mechanism of action. In addition, these biomarkers can be used to quantify and compare these properties (including paradoxical side-effects such as agitation in the case of benzodiazepines) between children and adults. For example, 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.

Finally, non-invasive monitoring of PK and PD could facilitate the evaluation of age-appropriate formulations of CNS stimulants and depressants for use in children, as illustrated by the studies described in chapters 8 and 9. Because oral delivery remains the most important and most commonly used route for drug delivery, suitable oral delivery forms that offer the possibility of customized dosing are urgently needed[40]. Sustained-release mini-tablets provide several distinct advantages over other conventional pediatric dosage forms; for example, mini-tablets can minimize dysphagia-related issues, they can be designed to mask unpleasant taste (thereby improving palatability and patient compliance), and they can be modified to release the active ingredient slowly[41]. In addition, mini-tablets can provide multiparticulate dosing, which is needed for global pediatric drug therapy[42]. In a recent study, mini-tablets were the best accepted oral formulation in infants and preschool children and were significantly more often fully swallowed than the other oral formulations[43]. Therefore, the new, age-appropriate prolonged-release melatonin mini-tablets (Circadin) described in chapter 8 may provide important advantages over the currently licensed 2-mg tablets. In this study, we evaluated the PK profile, short-term safety, and acceptability of this formulation in 16 children and adolescents with both autism spectrum disorder (ASD) and a sleep disorder. In order to avoid interrupting the subject's sleep due to repeated nighttime saliva sampling, the melatonin was administered in the morning (rather than in the evening); this also minimized study-related burden. Melatonin concentrations were measured non-invasively in saliva samples, and the level of sedation was evaluated using the Observer's Assessment of Alertness/Sedation (OAA/S) scale. This validated scale was originally developed to more objectively measure the level of alertness in sedated subjects, and it has been shown to be both reliable and sensitive to the effects of drugs such as midazolam[44]. In addition, the OAA/S scale is comparable to the Ramsey scale, which is commonly used in pediatric intensive care units to assess the patient's level of consciousness[45]. The concentration of melatonin in the saliva peaked within two hours of receiving either a dose of 2-mg or 10-mg Circadin, and the melatonin level remained elevated for several hours thereafter. Circadin exposure in saliva was dose-linear and clearance in saliva was comparable between dose groups. Sedation after the 2-mg Circadin dose peaked at 2 hours, which is around the $T_{max}$ time of the PK profile, demonstrating PK/PD correlation.

Non-invasive monitoring of an array of sensitive functional biomarkers can also be used to compare the effect profiles of different formulations, as was demonstrated in chapter 9. The 'proof-of-pharmacology' strategy described in this chapter 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 the study described in chapter 9, the pharmacokinetics of two doses of a novel highly concentrated aqueous intranasal midazolam formulation (Nazolam) was characterized in healthy adult volunteers and related to several outcome variables for sedation. In addition, the onset and duration of the pharmacological effect were evaluated and compared to intravenous midazolam using SPV, as a relationship between SPV reduction and clinical efficacy has been described[21]. Effects were seen on PD outcome variables of sedation and 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. This new formulation has potential for use in conscious sedation and epilepsy in children. A future study in children could include non-invasive measurement of midazolam in saliva[46] and repeated effect measurements on SPV (in older children) and a clinically relevant biomarker of sedation (OAA/S). In addition, a previously validated anxiety score (Faces Pain Scale-revised)[47] could be used to measure the effect on anxiolysis.

## FUTURE DIRECTIONS

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. The studies described in this thesis primarily involved adolescents,

as the development of non-invasive drug monitoring generally follows an incremental 'top-down' approach, with adolescents studied first, followed by younger age groups. Adolescents are often overlooked in clinical trials that lead to drug registration[48]. Changes in physical parameters such as height, weight, and lean body mass occur during puberty, and these changes can strongly influence hepatic drug elimination and clearance during adolescence. Age-dependent changes in liver size and/or functional capacity can also contribute significantly to changes in clearance[49]. In addition, many adolescents are more amendable to certain medications due to neurochemical differences, and the presence or absence of significant side-effects can strongly influence whether the subjects comply with the drug administration regimen[50]. Recent studies found that adolescence is a unique developmental period of transition in which specific brain regions undergo highly dynamic growth and pruning[51]. Changing the levels of catecholamine neurotransmitters can have the strongest effect during the transition from childhood to adolescence, when synaptic selection peaks[52]. Because adolescents are rarely waived for pediatric development under the Pediatric Regulation, this age group will likely be involved in more studies in the near future. However, preschoolers have historically been the most neglected age group with respect to psychopharmacological research[53], and this age group is also likely to be included in more trials performed under the Regulation. Currently, the youngest age at which a child is treated by a child psychiatrist is 2-3 years (e.g., in the case of a young child with irritability or aggressive behavior, for example in ASD). Children with disorders such as pain or epilepsy may also require drug therapy at a younger age. Importantly, younger children may be more sensitive than older children to the adverse effects of certain medications. Pre-school children with ADHD may have lower effect sizes with MPH, and they may have a higher prevalence of adverse events[54], including increased insomnia or other sleep-related problems, reduced appetite[55], increased emotional levels, social withdrawal, nausea, and stomach ache[56]. Therefore, age-appropriate non-verbal CNS tests that do not require a long attention span should be developed and validated in order to evaluate CNS effects in preschool children. In addition, relying upon the subject to spontaneously report adverse events is often not appropriate in this age group, and third-party reporting of adverse events is often needed[57]. Therefore, more proactive, objective measures must be taken, for example by incorporating the OAA/S scale as reported in chapter 8.

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 function and improved long-term outcome. To achieve this goal, the predictive value of putative functional CNS biomarkers should be investigated in long-term trials. Given that many neurological and psychiatric disorders are highly heterogenous, a combination of tests should be included in such a trial, as combined testing can yield a more precise and robust prediction of the drug's effect. These studies should also include tolerability biomarkers, given that adverse events can reduce compliance or even cause the subject to discontinue treatment.

Finally, as anesthetics and analgesics are among the most commonly cited therapeutic subgroups with a pediatric research need on the EMA Needs Lists[2] (as reported in chapter 2), more experience should be gained in the field of pediatric pain research, especially as children are particularly prone to unfair exclusion from pain research[58]. Pupillometry may be a useful non-invasive method to objectively quantitate pain response/intensity in children[59]. However, there are some important questions in pain and analgesia research that cannot be answered without an evoked pain stimulus, and therefore the use of evoked pain modalities should also be expanded for use in pediatric clinical trials. Evoked pain offers important control over the environment and provides standardization of pain stimuli, thereby enabling a more rigorous exploration of individual differences or the environmental factors that can affect the subjective pain experience. In general, it is ethically preferable to avoid causing pain for research purposes, especially in minors. In addition, because the induction of pain can lack direct benefit, it is usually not deemed appropriate for use in either therapeutic pediatrich research or non-therapeutic pediatric research. However, as the factors that can affect the

relationship between drug concentration and pain relief can differ between children and adults, findings obtained from adult studies may not necessarily translate directly to children or even adolescents. For example, a study of school-age children who underwent a tonsillectomy found that paracetamol can have a robust placebo effect in this age group[60] and a child's thoughts and attitudes regarding pain can change with age, thereby contributing to more intense feelings of pain during adolescence than in childhood[61]. In addition, developmental changes in endogenous analgesic mechanisms and developmental modulation have been proposed in animals[62]. Reaction time and late laser-evoked brain potentials decrease from childhood to adulthood, which may reflect aspects of maturation in sensory processing by the thermoalgesic system[63]. Because our current understanding of age-dependent effects and side effects is based largely on data collected from animal studies, evaluating age-dependent differences in PK and PD in pediatric clinical trials will be an important step forward. Therefore, it is necessary to include adolescents and children in pain studies. We predict that evoked pain will be used increasingly in children and adolescents. Therefore, additional knowledge and experience should be gained in the pediatric field of evoked pain modalities, again following an incremental 'top-down' approach, with adolescents studied first, followed by younger age groups.

### *Conclusions*

The clinical studies included in this thesis show that non-invasive drug profiling for CNS stimulants and depressants is feasible in healthy adolescents and children and adolescents 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.

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