Zopiclone and Traffic Safety

Introducing Legalized Blood Zopiclone Concentration Limits- Is it Evidence Based?

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Summary

Zopiclone is one of the most commonly prescribed sleep medications in the world. Driving in the morning, after regular nighttime zopiclone consumption, is, by many, considered to be safe traffic wise, due to the fast zopiclone elimination. Still, it is well known that the effects of zopiclone are comparable to that of benzodiazepines', and certain negative effects due to zopiclone intake, with respect to traffic safety, are therefore to be expected. This thesis aimed to investigate if zopiclone was suitable for implementing legal limits, by the use of blood zopiclone concentrations, in a manner similar to what is being conducted for ethanol in most countries. With the previous being plausible, an increased traffic accident risk was expected to be found related to the use of zopiclone, in addition to a positive concentration-effect relationship between blood zopiclone concentrations and traffic-related impairment, comparable to that of what has been found for ethanol.

We performed a coupling between the Norwegian Accident Registry (NRAR) and the Norwegian Prescription Database (NorPD), and found an increased traffic accident risk related to zopiclone exposure. There may, however, have been confounding factors present leading to a stronger relationship than what is actually true. Still, a significant traffic accident risk was found related to zopiclone exposure, in a case-crossover calculation, indicating a true drug effect.

An observational study design was used to investigate the relationship between high blood zopiclone concentrations and impairment, in a population of apprehended suspected drugged drivers, as assessed by the Norwegian by-the-road clinical test for impairment (CTI). A high share of impairment was found, increasing the higher the blood zopiclone concentrations. Similar results were found for ethanol.

Finally, a randomized controlled trial (RCT) was performed on 16 healthy volunteers. They were each given two different doses of zopiclone (5 and 10 mg), ethanol (50 g) and placebo, in a crossover design. The study found a positive concentration-effect relationship for zopiclone, as well as for ethanol. In addition, acute tolerance was found for zopiclone, as well as for ethanol. The relationship between blood zopiclone concentrations and blood alcohol concentrations (BACs) is found to be positive; however, there was some variation in response to the different impairment tests.

In total, the presented studies indicate that blood zopiclone concentrations may be as suited for legal limits as BACs.

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List of papers

Paper I

Gustavsen I, Bramness JG, Skurtveit S, Engeland A, Neutel I, Mørland J: Road Traffic Accident Risk Related to Prescription of the Hypnotics: Zopiclone, Zolpidem, Flunitrazepam and Nitrazepam. Sleep Med 2008; 9 (8) 818-822.

Paper II

Gustavsen I, Al-Sammurraie M, Mørland J, Bramness JG: Impairment Related to Blood Drug Concentrations of Zopiclone and Zolpidem Compared with Alcohol in Apprehended Drivers. Accid Anal Prev 2009; 41 (3) 462-466.

Paper III

Gustavsen I, Hjelmeland K, Bernard JP, Mørland J: Psychomotor Performance after Intake of Zopiclone compared with Intake of Ethanol– A randomized Controlled Double-Blinded Trial. J Clin psychopharmacol 2011; 31(4): 481-488.

Paper IV

Gustavsen I, Hjelmeland K, Bernard JP, Mørland J: Individual Psychomotor Impairment in Relation to Zopiclone and Ethanol Concentrations in Blood– A Randomized Controlled Double-Blinded Trial. Addiction 2012; 107(5):925-932.

Abbreviations

ATC	Anatomical Therapeutic Chemical
BAC	Blood Alcohol Concentration
BZ	Benzodiazepine
CFF	Critical Flicker Fusion
CI	Confidence Interval
CNS	Central Nervous System
CPT	Connors Continuous Performance Test
CRT	Choice Reaction Time
CTT	Critical Tracking Test
CTI	Clinical Test for Impairment
DEC	Drug Evaluation and Classification
DRUID	Driving Under the Influence of Drugs
DSST	Digit Symbol Substitution Test
DUI	Driving Under the Influence
DUID	Driving Under the Influence of Drugs
EMIT	Enzymatic multiplied immunoassay technique
EtOH	Ethanol
GABA	γ-amino butyric acid
GC	Gas Chromatography
h	hours
ICADTS	International Council on Drugs and Traffic Safety
LC	Liquid Chromatography
M	molar, used in µM
MS	Mass Spectrometry
N	Number
NCPR	Norwegian Central Population Registry
NIPH	Norwegian Institute of Public Health
NRAR	Norwegian Road Accident Registry
NorPD	Norwegian Prescription Database
RCT	Randomized Controlled Trial
R-enantiomer	R stands for rectus (Latin for right)
RR	Relative Risk
RT	Reaction Time
SDLP	Standard Deviation of Lateral Position
SEM	Standard Error of the Mean
S-enantiomer	S stands for sinister (Latin for left)
SD	Standard Deviation
SDS	Standard Deviation Speed
SIR	Standardized Incidence Ratio
SOC	Stockings of Cambridge
Z-hypnotic	Z stands for zopiclone, zolpidem and zaleplon
Zop	Zopiclone

Contents

0. Prologue

During the past 5 - 10 years, the Norwegian police have yearly collected breath- or blood samples from approximately 4,500 drivers suspected of driving under the influence (DUI) of alcohol. In addition, approximately the same number of drivers has yearly been apprehended due to the suspicion of driving under the influence of (non-alcoholic) drugs (DUID). More than 90 % of the blood samples collected test positive for one or more psychoactive drug, often revealing supra-therapeutic blood drug concentrations.

With regards to traffic cases involving alcohol, the Norwegian Traffic Act has declared a legal Blood Alcohol Concentration (BAC) limit of 0.02 %, with limits for more severe sentencing at 0.05 % and at 0.13 %; conveying that a higher BAC represent a more severe crime. Similar legal limits were drawn up, and implemented as of February 1st 2012, for 20 non-alcoholic drugs, in Norway.

Zopiclone, a medication used for treating insomnia, is one of the 20 mentioned non-alcoholic drugs. It is one of the most commonly prescribed drugs in Norway overall. Nearly 1/10 of the Norwegian population receive (at least one) zopiclone prescription each year.

1. Introduction

1.1 Traffic Related Impairment and Traffic Accident Risk

Driving a motor vehicle is a complex psychomotor task; to ensure a safe performance, the driver is required to occupy a broad range of skills and qualities. An estimated 90 % or more of traffic accidents may be linked to the driver.

All drivers have their own baseline level of energy and alertness. Every individual's baseline will naturally change due to e.g. aging, life situation and possible illness. A systematic review in 2005 found no evidence-based knowledge adept in determining medical fitness to drive. Furthermore, it has been found that drivers are unable to predict their own driving impairment [1].

Alcohol (ethanol) has often been used in experimental studies to induce traffic-related impairment. There are previously well documented negative effects of alcohol on required driving skills, in addition to an increased traffic accident risk, with an increasing concentration-effect relationship [2-4]. Alcohol is also the most commonly found drug among accident involved drivers [5]. Moskowitz and Fiorentino summarized in 2000 that BAC up to 0.10 % impair or influence negatively all of the following: dual attention, drowsiness, psychomotor skills, cognitive tasks, tracking, choice reaction time, vision, vigilance, perception, and simple reaction time [2]. A meta-analysis performed by Schnabel et al. concerning literature published between 1990 and 2007 reported similar findings [4]. It should be mentioned that each of the presented skills or tests may also be impaired by other factors. The impaired behavior caused by alcohol consumption is not necessarily similar to that of other causes of traffic-related impairment, like fatigue,

illness or non-alcoholic drug consumption [6]. However, alcohol-induced impairment is the best available objective, and most reproducible, factor when defining drug-related impairment relevant for traffic.

Non-alcoholic drugs have also been found to increase traffic accident risk, and to impair skills required for operating a motor vehicle. The previous has been observed in both epidemiological and experimental studies (7, 8). The evidence for traffic-related impairment by non-alcoholic drugs, however, is not yet as well established as it is for alcohol (6-8).

Different epidemiological study designs have analyzed and described the negative impact of psychoactive drugs on driving, both by roadside surveys and by traffic accident risk studies [7,8]. In summation, epidemiological studies have been able to find evidence of traffic-related impairment by benzodiazepines and (to some extent by z-hypnotics) [9-11], by cannabis [12], by amphetamine/methamphetamine [13] and by certain anti-depressants (for elderly people) [14].

Different psychomotor tests have been used in controlled experimental studies [15]. Some of these tests have an obvious correlation to real-life driving performance (high face validity), like the on-the-road standard deviation of lateral position (SDLP) studies [16] or vehicle simulator tests [17]. Other experimental studies have aimed at studying separate skills required for driving, similar to those described for alcohol. The number of different studies in the field is overwhelming, making it difficult to correctly compare results.

Table 1 The three recommended core levels of behavior to be measured during experimental drugged driving research. The table is cited from Walsh et al [8]

Behavior levels	Description	Examples
1. Automotive behavior	Well learned skills	Tracking, steering, vigilance or sustained attention
2. Control behavior	Maintaining distance, passing	Motor performance, maneuvers, divided attention, perception
3. Executive planning behavior	Interactive functions with ongoing traffic	Risk taking, impulsivity, information processing, attention, cognition, judgment

In order to systematize the compiling literature, several attempts have been made at categorizing the skills required for safe driving (15, 17). One of the latest guidelines was initiated by the International Council on Alcohol, Drugs and Traffic Safety (ICADTS) in 2007 [8]. This guideline gave specific recommendations for the different types of studies (experimental (behavioral) studies, epidemiology and toxicology). For experimental research, they recommended eight issues to be focused on in particular: 1) the use of psychomotor tests; 2) the choice of the study population; 3) the ethical and legal issues; 4) which drugs to be tested; 5) the specimens to correlate behavior impairment with drug levels; 6) the time interval for testing; 7) the issue of chronic use; 8) the choice of the study design.

Of special interest to the presented study, the ICADTS guidelines recommends three core levels of behavior to be measured during experimental research (table 1). The examples in the table below may be interpreted as a list of skills that should be well-executed to drive unimpaired.

1.2 Handling DUI

Based upon the well-established knowledge of an increased traffic accident risk related to the consumption of alcohol, most countries have, for several years, practiced legal limits of BAC within the range of 0.05 % - 0.10 % while driving. In Norway, there has been a legislative limit for BACs, as stated by the Norwegian Road Traffic Act, since 1936. The legal limit was changed from 0.05 % to 0.02 % in 2001, which is a low limit compared with most other countries [3,7].

The legal driving limit for BACs of 0.02 % is considered to be mainly a politically determined limit, meaning that there was no scientific proof, at the time of establishment, of traffic-related impairment for BACs this low. Due to the low legal limit, it has been shown that most Norwegian drives decide to stay completely sober when planning to drive [18]. In contrast, the (few) drivers who still drink and drive, often do so with a high BAC; the mean BAC of drivers apprehended due to a suspected DUI being approximately 0.15 % [19].

During the last few decades, there has been an international focus on to the problem of non-alcoholic drug use among drivers. Many countries have included the legal handling of DUID in their national laws. Legal handling of DUID may be impairment-based or based upon drug analyses alone [20].

Countries practicing *impairment-based* legislations will often assess traffic-related impairments using roadside tests, performed by a police officer or by a police physician, in addition to blood drug analyses. Since the 1970s there has been an increased focus on developing a systematic procedure for evaluating suspected DUI drivers [21]. A Drug Evaluation and Classification (DEC) program, first developed in California, has been spread and used across the USA and Canada, and has even been used in some parts of Europe and Australasia. The DEC program involves a series of procedures, including several psychomotor tests and toxicological analyses [21]. The Standardized Field Sobriety Test is included in the DEC program, and has a high predictability, at least for alcohol impairment [22]. Many countries use customized Clinical Tests for Impairment (CTIs), performed by physicians. Such CTIs are often more sensitive to alcohol impairment than to other possible drugs causing impairment [23,24].

Drug testing may be performed roadside, by using either a breathalyzer or immunological tests; or the driver can be apprehended and samples (usually being blood) may be collected for the analysis of possible impairing drugs. Some countries have introduced “zero tolerance” laws, or “low concentration limits”, to prevent the use of psychoactive drugs while operating a vehicle. This implies that the law must define legalized drug concentration limits, and any concentration above the given limits will induce a sentence,

no matter if the driver appears impaired or not. Some countries practice legislations that clearly differ between prescribed medications and an illegal use (i.e. without prescription). In these types of cases, driving under the influence of prescribed drugs will require evidence of impairment for providing a sentence, while driving under the influence of non-prescribed drugs will not [20].

In Norway, the police may request a blood test from any motor vehicle driver, at any point in time, on the suspicion of a drunk- and/or drugged driving. The police will decide in each case whether the blood should be analyzed for alcohol alone, or for a combination of alcohol and other possibly impairing drugs. The blood samples are analyzed by The Norwegian Institute of Public Health, (NIPH) Division of Forensic Medicine and Drug Abuse Research.

An *impairment-based* system is followed when sentencing non-alcoholic drug-related impairment by Norwegian law. Until February 2012 the results from the analyzed blood sample, in addition to the results from the CTI and the available information on drug use and possible illnesses, were used as a basis for conducting an expert statement in each individual case of suspected drugged driving. Such statements included a presentation on the likelihood of impairment at the time-point of driving, and an indicative comparison of the non-alcoholic drug-related impairment to a BAC level. The expert statements were used in court as a basis for sentencing [7]. The presented system resulted in a high detection rate of drugged drivers, but the procedure of individual evaluations was quite extensive and time-consuming. The decision of introducing legal limits for non-alcoholic drugs in Norway was therefore made (see Epilogue).

1.3 The Use of Hypnotic Drugs Related to Vehicle Driving

Drivers apprehended under the suspicion of being drugged, or drunk, have been found to have a high prevalence of hypnotic drugs in their blood [19,24-27]. Their blood drug concentrations have often been documented as supra-therapeutic, indicating drug abuse [24-27]. A Norwegian study from 1992 found that 90 % of the samples testing positive for benzodiazepines contained 2-3 drugs in the same sample, and that approximately 60 % of the samples showed illegal drugs in addition to the benzodiazepine(s) [26]. The most commonly detected benzodiazepine drug in the blood of suspected drugged Norwegian drivers has varied over the years, partly due to prescription rates [27,28] and availability on the illegal market.

Before starting work on Paper I, pharmacoepidemiological studies had already stated an increased risk of road traffic accidents related to benzodiazepine prescriptions, in particular for benzodiazepines with a long half-life [10,11,29-34]. The role of possible confounders in most of these studies was, however, unclear. Few epidemiological studies had investigated traffic accident risk in relation to measured benzodiazepine concentrations or to the size of prescribed doses [29,33]. A case-crossover study found no significant increase in traffic accident risk after a hypnotic exposure in general, but revealed an increased risk of traffic accident involvement related to zopiclone exposure alone [9].

After the introduction of zopiclone on to the Norwegian market in 1994, a gradually increasing number of motor vehicle drivers, apprehended under the suspicion of impaired driving, have tested positive for zopiclone. This increase corresponded well to the increasing sales rate [28]. Similar to that of benzodiazepines and illegal drugs, z-hypnotics were found in high blood drug concentrations among DUIs, indicating supra-therapeutic use [25]. The NIPH included zopiclone in the routine analysis from July 2001. Previous to July 2001, zopiclone was only analyzed on suspicion.

Table 2 Findings in the blood of apprehended suspects (mostly vehicle drivers) in Norway between 2007 and 2011 [19].

Year	Number of blood samples analyzed for non-alcoholic drugs	Positive[a] for zopiclone N (%)	Positive[a] for zolpidem N (%)	Positive[a] for nitrazepam N (%)	Positive[a] for flunitrazepam N (%)
2011	9676	149 (2 %)	66 (<1 %)	272 (3 %)	59 (<1 %)
2010	9597	135 (1 %)	52 (<1 %)	327 (3 %)	75 (<1 %)
2009	9657	158 (2 %)	70 (<1 %)	304 (3 %)	97 (1 %)
2008	9544	119 (1 %)	75 (<1 %)	371 (4 %)	201 (2 %)
2007	9122	125 (1 %)	64 (<1 %)	411 (5 %)	374 (4 %)

[a]Analytical cut-offs: zopiclone 0.05 µM, zolpidem 0.05 µM, flunitrazepam 0.1 µM, nitrazepam 0.1 µM, diazepam 0.2 µM, alprazolam 0.03 µM, oxazepam 1 µM, midazolam 0.2 µM, clonazepam 0.1 µM, and fenazepam 0.1 µM.

Even though z-hypnotics are commonly prescribed, it must be underlined that they are still not considered a main group of drugs to be found among apprehended persons suspected of being impaired [19,25] (table 2).

The share of zopiclone-positive drivers, among the population of standard Norwegian drivers, was reported based upon findings in the oral fluid of more than 10,500 randomly stopped drivers between 2005 - 2006 [18]: The study revealed that zopiclone was the single most frequently found drug, with as many as 1.4% of random Norwegian drivers testing positive for zopiclone. In comparison, 1.4 % of the drivers tested positive for any benzodiazepine, and 0.3 % tested positive for ethanol. It should be emphasized that these results not necessarily reflect impairment, but based upon the findings, a thorough investigation into the role of zopiclone in relation to traffic accident risk was desired.

1.4 Descriptions of the Drugs in Question

This thesis focuses on zopiclone.

Ethanol, in addition to the sleep medications: zolpidem, nitrazepam and flunitrazepam, has been used as comparator drugs in the different papers. Knowledge regarding traffic-related impairment for these drugs will therefore briefly be summarized.

Zopiclone has approximately 4/6 of the market share of hypnotics in Norway (calculated as financial turnover) [35]. Zolpidem, flunitrazepam and nitrazepam have approximately 1/6 of the market share, when calculated together. Other drugs may also be prescribed as

sleep medication, e.g.: melatonin, anti-histamines, anti-psychotics, anti-depressants, and herbal remedies. These drugs will not be further considered here.

In Norway, all prescribed drugs are classified into one of the following groups: A-, B-, or C-drugs; each with certain policies related to the classification group. Both A-drugs (e.g. morphine) and B-drugs (e.g. diazepam) are considered to have potential for abuse, B-drugs are considered to be weaker than A-drugs, while C-drugs (e.g. acetaminophen) are considered to have no potential for abuse.

Any prescribed drug, in Norway, known to possibly impair driving performance, has its packaging marked with a red triangle. This marking has been implemented by the authorities aiming to avoid drugged driving. All A- or B-drugs are marked with the red triangle, in addition to some drugs classified as C-.

1.4.1 Z-hypnotics: Zopiclone (and Zolpidem)

Z-hypnotics are benzodiazepine-like hypnotics with short elimination half-lives. Examples are zopiclone, zolpidem, and zaleplon. Zaleplon does not have a marketing authorization in Norway.

1.4.1.1 History

Zopiclone (marketed as e.g. Imovane® or Zimovane®) is the racemic mixture of R- and S-enantiomers, while eszopiclone (marketed as Lunesta® in the USA) only contains the active (S-) form.

Zopiclone was developed and introduced by Rhône-Poulenc S.A. (now part of Sanofi-Aventis) in the 1980s. It was promoted as a sleep inducer, and considered an improvement from benzodiazepines. The main arguments for the improvement were the faster elimination and the lower chances of residual effects, as compared with benzodiazepines. When zopiclone was originally introduced, it was as a racemic mixture only, with the strongest dose containing 7.5 mg of the active drug. In Norway, zopiclone was first introduced on to the market in 1994. It is now sold as 3.75 mg, 5 mg, and 7.5 mg tablets, under the names: Imovane® (Sanofi Aventis), Zopiklon® (Mylan), and Zopiclone® (Actavis).

Since 2005 the active stereoisomer, eszopiclone, has been marketed separately in some countries (e.g. USA), but not in Norway. The highest marketed dose of eszopiclone is 3 mg.

Zolpidem was introduced on to the Norwegian market in 1997. The drug is now sold in dosages of 5 mg and 10 mg tablets under the names: Stilnoct® (Sanofi Aventis) and Zolpidem® (Actavis).

Zopiclone and zolpidem are classified as B-drugs in Norway, and are marked with a red triangle.

1.4.1.2 Pharmacoepidemiology

Insomnia is considered to be present among 10 - 40 % of the adult population [36,37], and it is the only documented indication for prescribing z-hypnotics. Even though z-hypnotics are recommended for intermittent use only, meaning no longer treatment period than 2 - 4 weeks, they are often prescribed for longer time periods [37-39], and sometimes in even higher doses than those recommended [39].

Similar to what has been registered in other European countries [37] , the use of z-hypnotics has increased greatly in Norway since the early 1990s [40]. The share of the Norwegian population who had zopiclone prescribed at least once per year stabilized at around 7 % in 2007, and the percentage has remained the same since. Females constitute 2/3 of the users [40], and use is more common among the elder [39]. As many as 30 % of all females, in Norway, above the age of 80, had zopiclone prescribed at least once during 2009, compared with 21 % of all men above the age of 80 [38]. The higher use among the older females is probably related to a higher prevalence of insomnia among females compared with men [41], in addition to a higher prevalence of insomnia among the elder compared with younger people.

Z-hypnotics have taken over, and hold the largest share of the world-wide hypnotic drug market, during the past 1 - 2 decades [37,40]. The prescribing patterns seem to differ between countries, and even within a country [37,42], meaning that the “main” hypnotic drug prescribed will vary from place to place [37,43]. In the early 2000s, zolpidem was approximately two times as commonly prescribed as zopiclone, worldwide [43]. In Norway, zopiclone is prescribed more than six times as often as zolpidem [38].

Few studies have considered the abuse liability of z-hypnotic drugs. Due to the many similarities with benzodiazepines, some general awareness should be sought before prescribing a z-hypnotic drug to drug addict. A few reports concerning the abuse of zopiclone, or zolpidem, confirms a certain risk of abusing z-hypnotics, at least among people with a history of drug- or alcohol abuse. The abuse liability is, however, claimed to be lower for z-hypnotics than for benzodiazepines [43,44]. There is sparse information about an eventual illegal market for zopiclone. The Norwegian police has reported that 16 % of the approximately 32,000 incidents of illegal drugs seized in 2011 contained benzodiazepines [45]. Interestingly, z-hypnotics were not even mentioned in the report, indicating that z-hypnotics probably constitute a very low share of the Norwegian illegal market.

1.4.1.3 Pharmacokinetics

Zopiclone is administered orally as tablets. It is rapidly absorbed, with the C_{max} being reached within 0.5 - 4 hours after intake, and usually within 1 hour [46,47]. Bioavailability, after oral intake, is reported at around 80 % [47,48]. The C_{max} after the oral intake of 7.5 mg of zopiclone has been reported to be between 54 - 86 µg/L [47,49].

Patients with a liver- or a renal insufficiency, have been shown to have a higher C_{max} value [48]. About 45 % of zopiclone in plasma is bound to proteins [46].

Zopiclone is metabolized in the liver by oxidation and demethylation. The formation of N-oxide zopiclone (which has sleep inducing properties, though lower than the parent drug), and N-desmethyl zopiclone (which has some anxiolytic properties), is mainly metabolized by CYP3A4. In addition, CYP2C8 is involved in the formation of N-desmethyl zopiclone [50]. N-oxide zopiclone, N-desmethyl zopiclone, and unchanged zopiclone (<7 % of the dosage taken) are excreted via the urine [47]. The terminal half-life has been reported to be between 3.5 - 6.6 hours [47,49], and is severely prolonged for patients with liver failure and for elderly people [48]. Based upon the altered pharmacokinetics, older people, and patients with an organ failure, are advised to consume lower doses.

Concomitant treatment with CYP3A4 inducers (e.g. rifampicin) has been proven to reduce the blood zopiclone concentration [51], while concomitant treatment with CYP3A4 inhibitors (e.g. macrolides or grapefruit juice) may increase the blood zopiclone concentration [46]. The CYP2C8 inhibitor, gemfibrozil, has not been shown to increase the blood zopiclone concentration [46].

Clinical trials have found that an every day intake of 7.5 mg of zopiclone, for 14 days, does not significantly alter the C_{max} values. Only slight accumulations have been observed (34, 38).

Like zopiclone, zolpidem has a high bioavailability (70 %), and is metabolized by CYP3A4 [52]. Zolpidem has a terminal half-life of approximately 1.5 - 4.5 hours [49,53].

1.4.1.4 Pharmacodynamics

Zopiclone provide its effects by binding to the benzodiazepine receptors (ω or BZ), located on the γ-amino butyro acid $(GABA)_A$-receptor complex in the central nervous system. Two central benzodiazepine receptors have been identified: BZ_1 and BZ_2 [54,55]. The BZ_1- and BZ_2 receptors consist of different subunits: The BZ_1-receptor contains α1 subunits, while the BZ_2-receptors are heterogeneous and contain either α2, α3 or α5 subunits [56]. The binding to the subunit on the BZ-receptor mediates the specific effect. The BZ_1-receptor is known to be involved in mechanisms related to sleep- and wakefulness, while the BZ_2-receptor has been demonstrated to mediate cognitive-, anxiolytic-, memory- and psychomotor functions [54].

Zolpidem is found to bind specifically to the BZ_1-receptors [43,52]. Some researchers have claimed that zopiclone, like zolpidem, also binds specifically to the BZ_1-receptor, and thereby mediating less unwanted side effects compared with benzodiazepines [57]. The previous is yet to be verified in vivo [58,59]. Intake of zopiclone leads to much of the same effects as benzodiazepines: sleepiness/drowsiness, muscle relaxation, and amnesia, in addition to having anxiolytic- and anti-convulsive effects [49], with a liability for abuse [56]. It should be noted that animal studies have suggested that the BZ_1-

receptor is additionally involved in motor performance and in mediating abuse potential [54].

Sleep induction is the only indication for prescribing z-hypnotics. Zopiclone is proven to induce sleep, and maintain sleep quality, at dosages of 5 and 7.5 mg [49]. There are different opinions regarding zopiclone's residual effects (see Section 1.4.1.6). Some researchers claim that there is a low probability of residual effects if not exceeding the recommended dose of 7.5 mg [60,61]. Tolerance is reported to be unlikely [62,63]. However, there is evidence that long-term use of zopiclone, among patients suffering from insomnia, is non-effective in treating insomnia, and that cognitive therapy has a greater clinical effect for this group of patients [64,65]. The most common side effects reported for zopiclone are: bitter taste, dry mouth, drowsiness, and nightmares [49].

For zolpidem, a bed-time administration of the recommended dosage (5 - 10 mg) will not normally cause a residual sedation, nor impair the psychomotor performance during the following day [49,66]. The most common side effects are: dizziness, drowsiness, headache, and nausea. An increasing number of case report has related the intake of zolpidem to different incidents of parasomnias, describing complex behaviors like: sleep eating, sleep cooking, sleep driving etc. [67]. Although the long-term use of zolpidem is not recommended, several studies have found that zolpidem can maintain its effectiveness for up to several weeks [49].

A meta-analysis, aimed at comparing different hypnotic agents, did not find any convincing differences in wanted - or unwanted effects between zopiclone and zolpidem, nor between z-hypnotics and benzodiazepines [59].

1.4.1.5 Current Knowledge on Zopiclone and Traffic Accident Risk

Before commencing the presented PhD study, quite many epidemiological studies had investigated traffic accident risk related to benzodiazepine exposure [9-11,29-34]. In these studies benzodiazepines were investigated together, not differentiating between the specific drugs.

Barbone et al. performed a within-person case-crossover study in 1998, aiming to investigate tricyclic antidepressant drugs, benzodiazepines, selective serotonin-reuptake inhibitors or other drugs (mainly major tranquillizers); and reported an increased traffic accident risk for zopiclone and for anxiolytic benzodiazepines [9]. The N for zopiclone was, however, quite low, with only 14 traffic accidents related to zopiclone exposure. The results were still very interesting, in particular because the case-crossover design reduced the chance of confounding effects.

After publishing Paper I, other studies have found various degrees of increased traffic accident risk related to z-hypnotic exposure [13,68,69] (see Section 5.2).

1.4.1.6 Traffic Related Impairment: Current Knowledge from Experimental studies

Experimental Studies on Zopiclone Listed in the Appendix

The appendix displays 44 experimental studies (47 articles) on zopiclone and traffic-related impairment. These studies have been retrieved from literature search in Pubmed, MEDLINE and EMBASE, using relevant search words (as described in the Appendix), as of December 2011. Only objective tests on psychomotor impairment were considered. Papers inherent were not included.

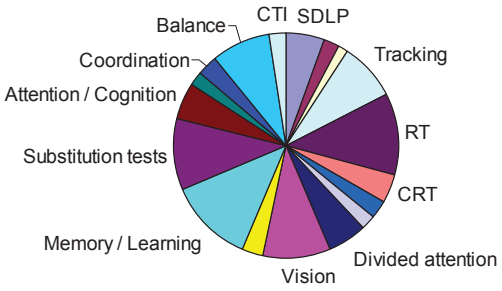


Figure 1: The frequency of use of the different tests included in the 44 experimental studies on the effects following zopiclone intake. Only the most commonly used tests are named at the figure

Healthy young volunteers were used as a study population in 34 of the 44 experimental studies (77 %). Only 4 studies were performed on patients suffering from insomnia [70-73]. Figure 1 shows the distribution of the different tests used in the experimental studies, as listed in the Appendix. The figure reveals that a wide range of tests have been used to

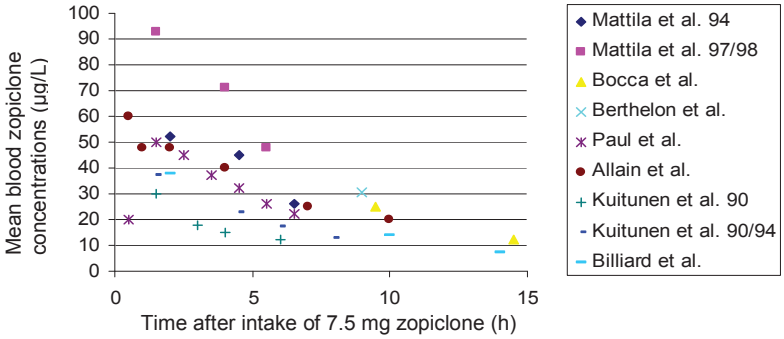


Figure 2: The distribution of measured blood zopiclone concentrations in previous RCTs after an intake of 7.5 mg of zopiclone. Mean values are shown for each group of healthy volunteers, related to time after intake. For the studies by Allain et al. and by Paul et al., the mean results were retrieved from figures

investigate zopiclone. Reaction time, tests related to learning or memory, and substitution tests were among the most frequently applied.

For 10 out of the 44 experimental studies, blood zopiclone concentrations were measured during the study [74-83]. One of these ten studies did not report the analyzed blood zopiclone concentrations in the article [83]. The remaining nine studies reported the mean blood zopiclone concentrations for the groups of volunteers. The measured mean values are presented in Figure 2. As illustrated, the mean blood zopiclone concentrations varied widely between the different studies, even though the zopiclone dose was 7.5 mg for all studies. In general, the measured blood zopiclone concentrations were lower, for many of the studies, than what would be expected from other studies focusing on pharmacokinetics. The Cmax following 7.5 mg of zopiclone has been reported to lie between 54 - 86 µg/L [47,49].

Table 3: A view of the different tests used for measuring impairment in the 44 experimental studies listed in the Appendix. The table illustrates how often the tests were not significantly impaired, and for studies demonstrating significant impairment: how long time after intake a significant impairment was documented. Only results after intake of 7.5 mg of zopiclone are included

Tests (Number of studies using the test)	Number of studies where the test was not significantly impaired after intake (%)	Number of studies where the test was impaired after intake related to the latest point-of-time after intake still demonstrating impairment (%):			
		> 0h ≤ 2h	> 2h ≤ 5h	>5h ≤ 9h	> 9h ≤ 13h
SDLP[a] (9)	1 (11%)				8 (89%)
SDS[b] (4)	2 (50%)				2 (50%)
Stop signal (2)	1 (50%)				1 (50%)
Tracking (14)	7 (50%)	3 (21%)	1 (7%)		3 (21%)
RT[c] (18)	8 (44%)	2 (11%)	3 (17%)	2 (11%)	3 (17%)
CRT[d] (8)	5 (63%)	2 (25%)			1 (13%)
Letter cancellation (3)	3 (100%)				
Errors (2)	1 (50%)				1 (50%)
Dual attention (8)	4 (50%)		1 (13%)		3 (38%)
Vision (16)	8 (50%)	3 (19%)	4 (25%)	1 (6%)	
Symbol copy test (5)	4 (80%)				1 (20%)
Memory/learning (18)	7 (39%)	3 (17%)	2 (11%)	1 (6%)	5 (28%)
DSST[e] (17)	6 (35%)	2 (12%)	3 (18%)	4 (24%)	2 (12%)
Attention/cognition (7)	2 (29%)	2 (28%)	1 (14%)	1 (14%)	1 (14%)
Tapping test (3)	2 (67%)				1 (33%)
Coordination (6)	4 (67%)		1 (17%)	1 (17%)	
Balance (12)	4 (33%)	3 (25%)	3 (25%)	2 (17%)	
CTI[f] (2)		2 (100%)			

[a]Standard Deviation of Lateral Position
[b]Standard Deviation Speed
[c]Reaction Time
[d]Choice Reaction Time
[e]Digit Symbol Substitution Test
[f]Clinical Test for Impairment

All 44 experimental studies related impairment to time after zopiclone intake, presenting mean impairment values for the groups of study populations. Significant impairment was defined as a significant difference between: mean performances after drug and placebo at

certain point-of-times after drug and placebo administration. Among the studies listed in the Appendix, none considered individual impairment, dichotomized as impaired or not impaired for each single observation.

Table 3 reviews different tests used in experimental studies on zopiclone effects in relation to time after intake still documenting impairment. Only the latest point-of-time, with significant impairment, was registered. Different time-points for measuring

Table 4: Studies relating BAC values to impairment following intake of 7.5 mg zopiclone

References	Used tests	Mean blood zopiclone concentrations compared to mean BACs	Time after intake of 7.5 mg zopiclone compared to time after intake of 0.3-0.8 g/kg ethanol (EtOH)
Kuitunen et al. 1990/1994 [23,78][b] 0.8 g/kg ethanol given. Mean blood drug concentrations measured at 1.5 h and 4.5 h after intake.	Attention, Body sway, Tracking errors, DSST and Vision	37 µg/L ≈ 0.08 % ~23 µg/L ≈ ~0.04 %	1,5 h zop ≈ 1,5 h EtOH 3 h zop ≈ 3 h EtOH 4,5 h zop ≈ 4,5 h EtOH
	Reaction time	37 µg/L > 0.08 % ~23 µg/L > ~0.04 %	1.5 h zop > 1.5 h EtOH 3 h zop > 3 h EtOH
	CTI	~37 µg/L < ~0.08 % ~23 µg/L ≤ ~0.04 %	2 h zop < 2 h EtOH 5 h zop ≤ 5 h EtOH
Mamelak et al. [71][a, b] 0.5 g/kg ethanol given.	Memory, RT, Tracking, DSST, Balance	(11 h zop) < 0.07 % (11 h zop) < 0.04 %	11 h zop < 1 h EtOH 11 h zop < ~2.5 h EtOH
Vermeeren et al. [84][a, b] Approximately 0.3 g/kg ethanol given.	SDLP	(10 h zop) > 0.04 %	10 h zop > 2 h EtOH
	Word learning, Tracking, Dual attention	(9 h zop) > 0.04 %	9 h zop > 1 h EtOH
Mattila et al. 1997/1998 [77,85] 0.65 + 0.35 g/kg ethanol given. Mean blood drug concentrations measured at 1.5 h, 4 h and 5.5 h after (the first) intake.	Tracking errors	~93 µg/L > ~0.08 % ~71 µg/L < ~0.09 % ~48 µg/L ≈ ~0.06	1 h zop > 1 h EtOH 3.5 h zop < 3.5 h EtOH 5 h zop ≈ 5 h EtOH
	Substitution tests	~93 µg/L ≈ ~0.08 % ~71 µg/L ≈ ~0.09 % ~48 µg/L ≈ ~0.06	1 h zop ≈ 1 h EtOH 3.5 h zop ≈ 3.5 h EtOH 5 h zop ≈ 5 h EtOH
	Reaction time	~93 µg/L >> ~0.08 % ~71 µg/L > ~0.09 % ~48 µg/L ≥ ~0.06 %	1 h zop >> 1 h EtOH 3.5 h zop > 3.5 h EtOH 5 h zop ≥ 5 h EtOH
	Body balance	~93 µg/L ≈ ~0.08 % ~71 µg/L ≤ ~0.09 % ~48 µg/L ≤ ~0.06 %	1 h zop ≈ 1 h EtOH 3.5 h zop ≤ 3.5 h EtOH 5 h zop ≤ 5 h EtOH
	Memory	~93 µg/L ≈ ~0.08 %	1.5 h zop ≈ 1.5 h EtOH

[a]Blood zopiclone concentrations not measured
[b]Blood alcohol concentration measured by breath test

impairment, and different tests applied, make it a complex task to sum up the overall test results. Some of the studies have aimed to investigate residual effects (often using sensitive tests), while some have aimed to investigate impairment during the first few hours after intake (often using less sensitive tests).

In 4 out of the 44 experimental studies, ethanol was used as a comparator drug for zopiclone impairment [23,71,77,78,84,85]. (The 4 studies were reported in 6 manuscripts.) Viewed together, these studies give an incomplete picture of comparable BAC values (Table 4).

DRUID Meta-Analysis

A meta-analysis of experimental studies on DUID research, as conducted between 1994-2006, was recently carried out [86], being part of the European DRUID program (Driving Under the Influence-Program). Impairment after oral drug intake, for each of the 33 possible impairing drugs, was related to time after intake, or related to blood drug concentrations. In cases where blood drug concentrations were not measured, an estimation based upon pharmacokinetic studies was performed. Drug impairment was further related to ethanol impairment based upon another DRUID meta-analysis on the effects of ethanol [4] (see section 1.4.2).

For zopiclone, 21 studies on the experimental effects on healthy volunteers were included in the meta-analysis. None of the studies in the DRUID report considered a higher zopiclone intake than 7.5 mg. The 21 experimental studies concluded on relevant impairment (higher than corresponding to BAC 0.03 %) up to 11 hours after the intake of 7.5 mg of zopiclone. Slightly more than 50 % of the effects measured at around 1 h after the intake of 7.5 mg of zopiclone were significantly impaired, corresponding to a BAC level of around 0.08 %. A higher percentage of the effects were significantly impaired at around 4 hours after intake. The method did not consider the sensitivity of the different impairment tests at the certain points-of-time after intake. Neither did the meta-analysis consider the matter of acute tolerance developing for zopiclone.

For zolpidem, the DRUID meta-analysis reported that more than 20 % of the effects were significantly impaired by 8 hours after the intake of 20 mg, and by 5 hours after the intake of 10 mg.

1.4.2 The Comparator Drugs: Ethanol, Nitrazepam and Flunitrazepam

Another meta-analysis, as part of the European DRUID program, was recently performed [4]. This report considered studies on ethanol published between 1990 and 2007. The meta-analysis aimed to provide a scientific basis in relation to traffic-related impairing effects, and to use the results as a reference function for the impairing effects of non-alcohol drugs in the DRUID meta-analysis on non-alcohol drugs. The report registered significant effects, related to BACs (measured or estimated values), on various

psychomotor tests, used in experimental studies. Nearly 3000 findings were reported, related to BACs between 0.01 % and 0.12 %. The meta-analysis confirmed previous knowledge on a positive concentration-effect relationship for ethanol, and found that simple tasks were less impaired than complex tasks, for low BACs. For high BACs, the complexity of the tasks did not matter [4]. Interestingly, the meta-analysis found no evidence for an acute tolerance development to ethanol. Based upon the DRUID meta-analysis for ethanol, it was estimated that BACs below 0.03 % corresponded to less than 15 % impaired observations, and that BACs above 0.08 % corresponded to more than 50 % impaired effects [86].

The report by Moskowitz and Fiorentino on BACs, found that some studies show significant impairment below 0.05 % BAC, most studies at 0.05 % BAC, and as many as 94 % of studies above 0.08 % BAC [2]. As expected, tests considered the most sensitive have shown impairment at low BACs, while less sensitive tests reveal impairment only at higher BACs. Driving, flying, and divided attention, all have been found sensitive (impairment even below 0.01 %), while tests such as simple reaction time and critical flicker fusion test (CFF) have been found less sensitive. Ethanol is also proven to be a cause of traffic accidents, in a positive concentration-effect relationship [3]. All in all, ethanol is therefore considered feasible as a positive control in experimental DUID research [8].

Flunitrazepam and nitrazepam are benzodiazepine hypnotics marketed in Norway; both having long terminal half-lives. It has been documented that benzodiazepines, in general, are possible impairing drugs, also commonly abused among polydrug users [37]. Flunitrazepam has received some negative attention because of abuse of Rohypnol [87,88]. Based upon the negative attention, and the police's disclosure of illegal import, Rohypnol was made an A-classified drug in 2003 in Norway, markedly lowering sales rates and findings of the drug in the blood samples from suspected drugged drivers [19]. The manufacturer decided to withdraw Rohypnol® from the market in 2004 [89].

Nitrazepam has been considered a less "dangerous" drug, although there is, in fact, no evidence of such a difference based upon the pharmacological properties. A recent Norwegian study found that nitrazepam was the benzodiazepine most often prescribed in conjunction with other benzodiazepines [90].

The recent DRUID meta-analysis on non-alcohol drugs followed a similar design to the DRUID ethanol meta-analysis [86]. The meta-analysis aimed to investigate possible traffic-related impairment for 33 possibly impairing drugs. For flunitrazepam, a linear relationship between percentage of impaired effects and (estimated) blood flunitrazepam concentrations was found. For nitrazepam, the findings were not equally clear. Former studies have, however, found evidence of traffic-related impairment following nitrazepam, as well as flunitrazepam, based upon both experimental- and observational studies [7].

2. Aims

The aim was to investigate the scientific basis for introducing legal limits for zopiclone related to traffic. A scientific basis was postulated to include: a) demonstration of an increased traffic accident risk related to the use of zopiclone, and b) a positive concentration-effect relationship between blood zopiclone concentrations and traffic-related impairment, comparable to what had previously been found for ethanol.

We aimed at further exploring the following three questions:

2.1 Aim 1

Does use of zopiclone increase traffic accident risk? (Paper I)

2.2 Aim 2

Is there a positive concentration-effect relationship between zopiclone concentrations and traffic-related impairment? (Papers II, III and IV)

2.2 Aim 3

Are there any fundamental differences between the concentration-effect relationships (as mentioned under Aim 2) for zopiclone and for ethanol? (Papers II, III and IV)

3. Material and Methods

3.1 Paper I

3.1.1 Study Design

Paper I is an observational study. We used a cohort design.

3.1.2 Sources

Three sources of data were used: the Norwegian Prescription Database (NorPD), the Norwegian Road Accident Registry (NRAR), and the Norwegian Central Population Registry (NCPR).

The NorPD is a research database that captures all dispensed prescriptions from Norwegian pharmacies as of January 2004 [91]. The database only contains information on prescriptions in relation to ambulatory treatment; it does not include prescriptions given to hospitalized patients. As an example, in 2007, 68 % of the Norwegian population were registered as having dispensed at least one prescribed medicine [92]. The registry includes information on the patient (pseudonymous identification numbers, their residence etc.), the prescriber (their speciality, their gender, their identification number etc.), the drug (the ATC (Anatomical Therapeutic Chemical) code, the dose, the number of tablets etc.), and the pharmacy dispensing the drug (the county in which it is placed etc.) [92]. Pharmacy records of dispensed drugs are electronically transferred to NIPH through Statistics Norway to ensure confidentiality. Statistics Norway replaces both the patient's identification number and the prescriber's identification number with pseudonymous numbers.

The NRAR provides information on motor vehicle accidents involving personal injuries on Norwegian roads [93]. Any traffic accident with a personal injury in Norway is required to be registered by the police, who report to the NRAR. NRAR does not provide information as to whether the driver was responsible for the accident, nor as to the severity of the injury. Less severe accidents and injuries are often not reported to the police, and will therefore remain unregistered by the NRAR.

The NCPR contains information on all Norwegian inhabitants, e.g. their name and their unique identification number, as assigned to each individual living in Norway. Unique identification numbers allows an assured coupling between the registries. The NCPR is administered by the Norwegian Directorate of Taxes.

3.1.3 Study Population

Paper I studied the entire Norwegian population aged 18 - 69 during the time period: January 2004 - October 2006 (including 3.1 million people). The population was stratified into groups by age and gender.

3.1.4 Exposure

Paper I defined hypnotic exposure as having dispensed a hypnotic prescription for one of the following drugs: zopiclone, zolpidem, nitrazepam or flunitrazepam; further differentiating between the first 7 days and the first 14 days after dispensing, where the first day was defined as the day after the date of the dispensation.
SIR was calculated in different ways:

a) Concurrent prescriptions for other medications were not considered
b) Those with concurrent additional psychoactive drug prescriptions were excluded
c) Only incidental hypnotic drug users were included (180 day washout)
d) Only drivers, who, during the study period, had been involved in accident(s), as registered in the NRAR, were considered (case-crossover: results not shown in Paper 1)

3.1.5 Outcome: Standardized Incidence Ratio (SIR)

The SIR is the ratio between the number of traffic accidents in the exposed person-time and the non-exposed person-time (Figure 3).

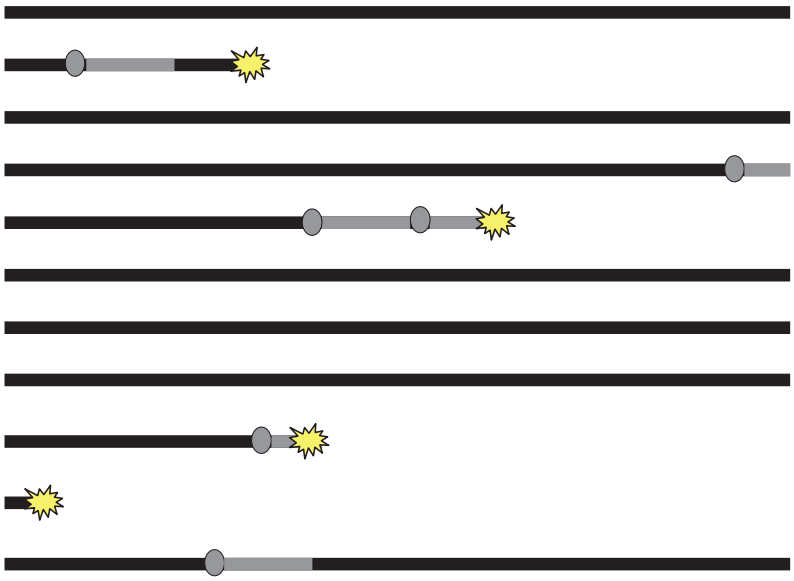


Figure 3: An illustration of the exposed- and non-exposed person-time. Each line represents an individual. The stars represent traffic accidents and the oval circles represent the subject dispensing one of the hypnotic drugs in question. The oval circles are followed by grey lines, representing exposed person-time. The black lines represent non-exposed person-time

3.2 Paper II

3.2.1 Study Design

Paper II is an observational, cross-sectional study based upon an internal data source at the NIPH.

3.2.2 Sources

The study is based upon the Apprehended Driver Registry located at the Division of Forensic Medicine and Drug Research. Since December 2000 these data have been stored in a computer program by the name of StarLIMS, which is also an integrated laboratory management system (STARLIMS Corporation, 4000 Hollywood FL 33021). Approximately 82 % of the cases requested by the police to be analyzed are apprehensions due to suspicious driving or due to traffic accident involvement (data retrieved from StarLIMS Database regarding the years 2007-2011). The remaining 18 % constitute cases involving other types of crimes, such as assault and battery.

The CTI results, and the analytical results, were retrieved from the StarLIMS database. StarLIMS contains information on all cases where the Norwegian police request a blood drug analysis due to a suspected criminal offence. The StarLIMS database contains information on the incident (e.g. the place, the time point, the reason for the requested blood sampling), the suspect (e.g. the name, the gender), the analytical results, and information on- and the outcomes of the CTI. A standardized procedure, related to forensic toxicology, was thoroughly followed with regards to the chain of custody and the analytical procedures for all of the included blood samples.

3.2.3 Study Population

The population of drivers and other apprehended criminal suspects, during the time period between 2000 and 2007, where the police requested blood drug analyses (N = 35,756), were investigated. For practical reasons, the study population was called apprehended *drivers* throughout Paper II. Former research has shown that this population includes for the most part a marginalized subpopulation of Norwegian drivers, with high blood drug concentrations and with high DUI recidivism rates [94] and mortality [95].

3.2.4 Exposure

The blood samples were screened for the most common non-alcoholic drugs of abuse, in addition to alcohol. Samples revealing other drugs than zopiclone, zolpidem or ethanol, as confirmed in blood, and samples containing more than one drug, were excluded.

The screening used a combination of enzymatic- (alcohol), enzyme multiplied immunoassay technique (EMIT)-, and liquid chromatography / mass spectrometry (LC/MS) methods. Approximately twenty-five potentially impairing non-alcoholic drugs were routinely screened for, including: amphetamines, a number of benzodiazepines, cannabis, cocaine, and opiates. In addition, carisoprodol, meprobamate, methadone,

carbamazepine, and phenobarbital were routinely screened for in samples received after May 2001. Z-hypnotics were routinely screened for in samples received after June 2001. All positive screening results were confirmed.

The confirmation analyses were performed by LC/MS for zopiclone and zolpidem, and by headspace gas chromatography (GC) for alcohol [96]. The cut-off value for zopiclone was 19 µg/L, 15 µg/L for zolpidem, and 0.004 % for ethanol.

3.2.5 Outcome; Impairment

In those cases where the police suspect a driver of being under the influence of non-alcoholic drugs, a police physician will perform a CTI shortly after the apprehension. The Norwegian CTI consists of three separate parts: First, the physician obtains information on current and former drug use, in addition to any other type of information which may explain the suspected impairment (e.g. diseases, disabilities etc.). Second, the physician will instruct the apprehended to perform a set of subtest, recording the results on a standardized form. Examples of subtests are: motor coordination, cognitive functions, and alertness. In addition, the physician must evaluate certain signs, such as: appearance. All together, the CTI includes twenty-five subtests and signs [97]. Third, the physician must make a conclusion as to whether the apprehended appears drug impaired or not impaired (selecting between five choices: not impaired – slightly impaired – moderately impaired – highly impaired – not possible to conclude on impairment). The conclusions are dichotomized in Paper II to: “impaired” or “not impaired”.

The Norwegian CTI is performed by different local physicians, some of whom rarely administering the test. The CTI was originally created to reveal alcohol impairment, but has later been modified to cover some of the signs of central nervous system (CNS) stimulant- or depressant consumption (e.g. time-perception and restless appearance). Positive relationships between CTI assessed impairment and blood drug concentrations have been documented for ethanol [24,98], carisoprodol [99], codeine [100], Δ-9-tetrahydrocannabinol [101], (meth-)amphetamine [102], and morphine/morphine-6-glucuronide [103]. In accordance with previous literature on impairment [3,23], the strongest relationship between blood drug concentrations and impairment, as assessed by the CTI, is documented for ethanol.

3.2.6 Data Processing

3.2.6.1 Data Programs

Statistical analyses were calculated using Excel version 2003 and SPSS versions 14.

Sigmaplot version 9 (SYSTAT software Inc.) was used to make figures.

3.2.6.2 Statistic tests

A Pearson's test was used for calculating differences between the shares of impaired observations related to the different drug concentration. Fisher's Exact test was used for calculating differences when the number of observations was low.

Mann Whitney U-test was used for calculating differences between the groups.

A binary regression model was used, where impaired was the dependent variable, and blood drug concentration, age, and gender all were independent variables. 95 % CI (confidence intervals) were used.

3.3 Paper III and IV

3.3.1 Study design

A double blind, randomized, controlled trial (RCT) was used to investigate 16 volunteers with respect to the effects of zopiclone and ethanol, in a 4-way crossover setup.

3.3.2 Study Population

A small pilot was performed in advance of the actual study to determine the number of required volunteers. The pilot included two volunteers, which were each given 50 g of alcohol. The volunteers performed only the Connors Continuous Performance Test (CPT) test, because standard errors of measurements for different age-groups and gender were already provided in the CPT manual. The values obtained from the different test components were used to calculate the standardized difference based upon the Altman's nomogram, with $P=0.05$ and 80 % power: The results indicating it necessary to include at least 10 test subjects in the study, this allowing the determination of a true differences for at least two CPT test components. Consequently, an attempt was made at recruiting 15 - 20 volunteers.

16 healthy male volunteers with a median age of 23.5 years (range 20 - 28), and a median body weight of 76.5 kg (range 69 - 88), were included in the study. The volunteers were required to refrain from alcohol consumption during the final 72 hours before each session, in addition to refraining from any type of medication during the preceding week.

Exclusion criteria were:

1. History of- or current drug/alcohol abuse
2. Former abnormal reaction to any type of hypnotic drug
3. Intake of zopiclone during the 3 months previous to the study
4. Regular (daily) intake of any prescribed drug
5. History of severe allergic disease
6. History of significant mental, cardiovascular, renal, or hepatic disorder, or any other significant disease as judged by the investigator

7. Positive pre-session urine sample (confirmation analysis) for any of the following substances: ethanol, benzodiazepines, zopiclone, zolpidem, tetrahydrocannabinol, cocaine, amphetamine, methamphetamine, morphine or codeine

3.3.3 Study Medications

Four different study medications were provided for the RCT: 5 and 10 mg tablets of zopiclone, 50 g of ethanol and placebo. The manufacture, the blinding procedure, and the packaging of the studied drugs are described in detail in Paper III.

Figure 4: Study medications: two capsules and one liquid drink were given to each subject, each morning, on each study day

Preceding the study, another small pilot was conducted using four volunteers. The aim was to detect the expected blood zopiclone concentration levels, after the intake of zopiclone, at the previously decided time-points for blood sampling. Another aim was to investigate if blood zopiclone concentration levels differed after capsule- or original tablet intake. Lower concentrations than expected was found for zopiclone in blood, after the consumption of both capsules and tablets, as compared with results from former studies [47]. No difference was found between blood zopiclone concentrations with capsular- and original tablet form.

3.3.4 Blood drug analyses

The quantifications of zopiclone and ethanol in blood were performed by HPLC-MS, and by headspace GC analysis, respectively (see Paper III). The limits of quantification were 7 µg/L for zopiclone and 0.004 % for ethanol.

All blood samples were stored at 4 ºC immediately after sampling. Analyses were started within 24 hours.

3.3.5 Tests

The aim was to use psychomotor tests that covered all three levels of behavior; Automotive, Control and Executive Planning Behavior [8]. In addition, the tests had to suit the study design, the localities and the time frames. A description for each of the selected tests is presented in Table 5.

Table 5: Tests chosen for the RCT

Test	Short description	Test duration	Explanation for the choice
SOC	Measures executive planning and motor control	10 min	Described as quite similar to the test by the name of: Tower of London, which is used in DUI studies
CRT	Measures response speed and ability to make correct decisions quickly	7 min	Well known and used in DUI studies
CPT	Measures attention: clinically used among attention deficit hyperactivity disorder patients	14 min	Quite long duration. Appeared sensitive to drug effects

3.3.6 Assessment of Behavioral Levels

Twenty-three test components (4 + 7 + 12), from the three selected computerized tests, were available. The aim was to include representative test components, and not to use any measured parameter more than once (e.g. some test components were registered as both median and mean). The descriptions of each test component, as given in the test manuals, were used as a basis for the selection.

Fifteen test components were deemed appropriate and categorized into three behavioral levels, based upon the descriptions given in the test manuals related to the Talloire report [8] (Table 6).

3.3.7 Assessing Impairment

Paper III used placebo as a reference when calculating possible impairment. Mean values were calculated, at each point-in-time examined after intake, for each of the single test components. Any significant deterioration between the mean performances, as compared with placebo, was defined as impairment.

Paper IV dichotomized each of the volunteer's single psychomotor test performances, after being compared with the volunteer's own baseline performance, into either impaired or not impaired. The baseline performance was defined for every single test component, for each of the 16 volunteers. The values were given by using the range of four baseline performances, and adding/subtracting 5 % off the mean baseline test performance. Any test result above the range of individual baseline performance +/- 5 % was defined as impaired, while any test result similar as, or below, the range of individual baseline performance +/- 5 %, was defined as not impaired.

Table 6: A description of all of the included test components, divided by behavioral levels

Level 1: Automative behaviors [a]	
CRT rt var	The standard deviation of the reaction time. Measured consistency of reaction time
CPT rt var	The standard error of reaction time. Measured consistency of reaction time
CRT omis	Omissions: the number of targets to which the volunteer did not respond. Measured automative behavior
CPT omis	Omissions: the number of targets to which the volunteer did not respond. Measured automative behavior
CPT alert	The slope of change in reaction time over the 6 blocks. Measured the ability to stay alert. A vigilance measure
CPT adjust	The slope of change in reaction time related to the inter stimulus intervals. A positive slope indicated a slower reaction time as the inter stimulus interval increased. Measured the ability to adjust to the presented speed
Level 2: Control Behaviors [a]	
SOC r time	Reaction time: the volunteers' speed of movement from initial move to last move for the 5 moves-problems
CRT r time	Reaction time: the mean latency from stimulus appearance to button press
CPT r time	Reaction time: the mean response time for all 6 blocks
CRT pers	Perseverations: the percentage of trials the volunteer responded too fast. Measured perception and motor performance
CPT pers	Perseverations: the number of times the volunteer responded too fast. Measured perception and motor performance
Level 3: Executive Planning [a]	
SOC plan	The mean time to select the first ball in the 5 moves problems. Measured planning and cognition
SOC incor	How many times the volunteer not completed the problems in the minimum possible number of moves. Measured information processing, attention and cognition.
CRT com	Commissions: the percentage of trials the volunteer pressed the wrong button. Measured information processing and attention
CPT com	Commissions: the number of times the volunteer responded to a non-target. Measured impulse control and attention

[a] [8]

3.3.8 Data Processing

3.3.8.1 Data Programs

Statistical analyses were calculated using Excel version 2003 and SPSS versions 17.

Sigmaplot version 12 (SYSTAT software Inc.) was used to make figures.

The software belonging to the computerized tests SOC, CRT and CPT converted the results from each study day into Excel tables. SOC and CRT are licensed from CANTABeclipse™ (Version 3, © 2006, Cambridge Cognition Ltd.). CPT is licensed through Multi-Health Systems Inc. (© 2000, 2004).

StarLIMS management system was used in the process of receiving, analyzing and storing the blood (and urine) samples (see 3.2.2). The volunteers were identified by tracking numbers only; no names or birthdates were registered in the StarLIMS system.

3.3.8.2 Statistic tests

Paper III used a paired sample analysis (Wilcoxon test) when calculating the difference between mean performance after intake of each active drug and mean performance after placebo intake.

A Pearson's test was used for calculating differences between the shares of impaired observations related to the different drug concentration groups, in Paper IV. Fisher's Exact test was used for calculating differences when the number of observations was low, in Paper IV.

3.6 Ethical Considerations

The data included in Paper I is coupled by each individual's unique 11-digit identification number. Permission to perform the coupling was given by the Norwegian Data Inspectorate before the study was conducted.

All the data used in Paper II were handled anonymously, meaning that names and identification numbers were replaced by a tracking number. The use of internal statistics was interpreted as a part of regular routine management, precluding the need to apply for permission before performing the study.

The experimental study was approved by both the Regional Ethical Committee for Medical Research and by the Norwegian Medicines Agency. The trial was conducted in accordance with the Helsinki Declaration. All volunteers were given thorough information on the study before enrolment, and volunteers were only enrolled after having given a written consent. The volunteers were offered compensations of 4000 NOK after completing all four days of the study. The volunteers were not registered by their personal names, but were given each a specific tracking number throughout the trial.

- We included only male volunteers. Due to hormonal fluctuations, such as possible pregnancies and the common use of contraceptives, females are, in general, less desired as research objects. As a consequence, the evidence-based knowledge is less powerful for females than for males. Only male volunteers were studied to avoid the possible challenges of female volunteers, and because males are more often involved in DUI.
- There is always a certain level of risk involved when healthy individuals receive drugs intended for pathologic conditions. Inducing drug- and alcohol impairment may be considered an even higher risk. The impression left by the volunteers, after participating in the study, was that the study revealed, and gave attention to, the negative impairing effects of zopiclone and ethanol. Only volunteers with no history of (self-reported) drug abuse were included in the study.

With all the studies, short Norwegian summaries were submitted to the press after publication, leading the studies to receive a certain level of attention in the Norwegian media. As a result, some claimed that patients suffering from insomnia were stigmatized, leading to an overstated fear of the effects upon zopiclone consumption, and that driving without taking the prescribed medicine could be even more hazardous. Our results were correctly referred to by the press, and it was clear that we did not compare traffic accident risk among insomnia patients who had taken their prescribed medicine and those who had not. One may argument that we, as well as any other researcher, had an ethical obligation to inform the public about our findings.

4. Summary of Results

4.1 Aim 1: Traffic Accident Risk Related to Zopiclone Use

Paper I revealed an increased traffic accident risk after dispensing prescriptions for all of the investigated hypnotics. The SIRs (95 % confidence interval (CI)) and the observed number of accidents are presented in Table 7. Calculations made, based upon a time period of 14 days after dispensing, gave slightly lower risk values for all of the hypnotics, as compared to 7 days of exposure. Excluding the individuals concurrently receiving other psychoactive drugs gave a SIR of 1.9 (1.5 - 2.4) and 2.4 (1.2 - 4.2), for zopiclone and nitrazepam respectively. Dispensing a zopiclone prescription after a 180-day washout gave a SIR of 2.1 (1.3 - 3.1). None of the other hypnotics included had a sufficient number of accidents to calculate incidental use. The case-crossover calculations, including only the drivers involved in accidents lowered the SIRs for all of the hypnotics, although significant results were still found for zopiclone and nitrazepam (only). The case-crossover results were not shown in Paper I, but included in table 7 below.

The highest SIRs were found among the youngest age groups for all of the hypnotics. In general, males had higher SIRs than females, in all age groups. The largest and most consistent difference between males and females was observed for flunitrazepam.

Table 7: The SIRs and the corresponding number of accidents during the observation period, for the four investigated hypnotic drugs. SIRs were not calculated when the number of accidents (N) was below 10

	Zopiclone			Zolpidem			Nitrazepam			Flunitrazepam		
	N	SIR[c]	95 % CI	N	SIR	95 % CI	N	SIR	95 % CI	N	SIR	95 % CI
7 days of exposure[a]	129	2.3	2.0-2.8	21	2.2	1.4-3.4	27	2.7	1.8-3.9	18	4.0	2.4-6.4
14 days of exposure[a]	204	2.0	1.7-2.2	38	2.1	1.5-2.9	41	2.2	1.6-3.0	25	3.1	2.0-4.6
Concurrent users excluded[b]	80	1.9	1.5-2.4	<10			11	2.4	1.2-4.2	<10		
Incidental users only[a,b]	22	2.1	1.3-3.1	<10			<10			<10		
Case-crossover[a,b]	129	1.4	1.2-1.7	21	1.1	0.7-1.7	27	1.7	1.1-2.5	18	1.5	0.9-2.4

[a]Concurrent prescriptions for other medications were not considered
[b]7 days of exposure
[c]Standardized Incidence Ratio

The mean age of the drivers involved in the car crashes exposed to zopiclone was slightly higher than the mean ages for the other hypnotics (46 years for zopiclone versus 40 for zolpidem, 39 for nitrazepam, and 38 for flunitrazepam).

4.2 Aim 2: The Concentration-Effect Relationship between Zopiclone and Impairment

Papers II, III and IV revealed positive concentration-effect relationships between blood zopiclone concentrations and traffic-related impairment, by using both different test methods and different blood zopiclone concentration levels.

Only 9 % of the 79 individuals included in Paper II had blood zopiclone concentrations corresponding with a therapeutic intake (Group 1). The remaining all had higher blood

zopiclone concentrations than those likely following therapeutic use. In contrast, Papers III and IV only included moderately high blood zopiclone concentrations, few exceeding the values of Group 1 in Paper II.

In paper II, 71 % of the drivers in Group 1 were considered to be impaired (19 - 32 µg/L), 86 % in Group 2 (33 - 129 µg/L), and 97 % in Group 3 (130 µg/L or more). The increasing share of impaired drivers related to the increasing blood zopiclone concentrations was only significant between Groups 2 and 3. The lack of significance between Groups 1 and 2 is most likely due to the low number of drivers in Group 1.

Paper III aimed to demonstrate mean psychomotor performance related to time after intake, and documented impairment at all three behavioural levels, comparing with placebo. The most pronounced impairment was seen for automotive behaviour. For all behavioural levels, impairment was greatest around one hour after intake. The mean blood drug concentrations observed one hour after intake were: 39 (+/-SEM 4) µg/L for 10 mg of zopiclone, 19 (+/- SEM 2) µg/L for 5 mg of zopiclone, and 0.07 (+/-SEM 0.003) % for BAC.

An intake of 10 mg of zopiclone tended to show greater impairment than 5 mg of zopiclone for all behavioural levels, but only 4 out of the 15 test components demonstrated a significant performance difference between the two doses. Two of the test components were still able to show impairment 3.5 hours after intake of 10 mg of zopiclone, as compared with placebo. The mean blood zopiclone concentration at 3.5 hours after the intake of 10 mg of zopiclone was 34 (+/-SEM 2) µg/L. Less impairment was found at 3.5 h after the intake of 10 mg of zopiclone (34 µg/L) compared with at one hour after the intake of 5 mg of zopiclone (19 µg/L), indicating some level of acute tolerance.

Paper IV found a positive relationship between blood zopiclone concentrations and percentages of impaired observations, both for automotive behaviour and control behaviour. The positive relationship for blood zopiclone concentrations started at >16 µg/L. No such relationship was found for executive planning behaviour. Furthermore, the curve obtained for blood zopiclone concentrations shortly (<1 hour) after intake was more vertical than the curve obtained later (>1 hour after intake). The positive relationship was significant from 1 µg/L and upwards. For blood samples collected >1 hour after intake, slightly more impaired observations were made, in correspondence with higher blood drug concentrations. Observations made more than one hour after the intake of zopiclone showed significant impairment above 25 µg/L, indicating that there is a positive relationship between impairment and blood zopiclone concentrations, also with investigations long after intake.

4.3 Aim 3: Impairment, Observed at Different Blood Zopiclone Concentrations, Expressed as BAC

Comparisons were made between performances at different blood zopiclone concentrations and different BACs, and are presented in Figure 5.

As revealed in the figure, Papers II and IV documents that any measureable blood zopiclone concentration up to 20 - 30 µg/L is comparable to BACs at around 0.05 %. For blood zopiclone concentrations raging 30 - 40 µg/L, and up to 74 µg/L, a comparison can be made to BACs between 0.05 - 0.10 %.

Only Paper II includes blood zopiclone concentrations above 74 µg/L.

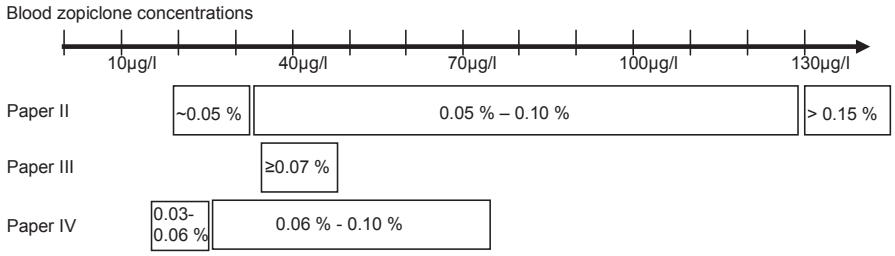


Figure 5: The relationships found between blood zopiclone concentrations and BACs in Papers II – IV

In both Paper II and IV, zopiclone and ethanol seem to follow an overall similar response to the psychomotor tests. In Paper II, both drugs demonstrate a high chance of impairment (>70 % of the observations) among the lowest blood drug concentrations, gradually increasing to nearly 100 % impairment among the individuals with the highest blood drug concentrations. In Paper IV, results were calculated for each of the three behavioural levels, not showing results for each single test component. The results demonstrate a positive concentration-effect relationship, both for ethanol and for zopiclone, in relation to automotive behaviour and control behaviour, but not for executive planning behaviour.

Paper III focuses on the test component results, and was able to demonstrate a different response pattern for ethanol compared with zopiclone. Ethanol was found to increase the chance for errors, while zopiclone led to a slowed response, an impaired alertness, and a less consistent performance.

In Paper IV, acute tolerance was documented both for ethanol and for zopiclone. Zopiclone shows a clearer positive concentration-impairment relationship, also among the blood samples collected >1 hour after intake. The lack of a positive concentration-effect relationship for ethanol may be explained by the low number of ethanol cases compared with zopiclone (double N for zopiclone).

5. Discussion

5.1 Methodological Considerations

5.1.1 Paper I

The entire Norwegian population aged 18 - 69 was included in the study. Use of nationwide registries gave a unique opportunity to study a large amount of material. Given that NorPD, NRAR and NCPR all were correctly updated, there were no information biases.

The presented study considered only prescribed drugs, and did not register concurrent use of alcohol or illegal drugs. Alcohol, and to some extent illegal drugs, are considered well known causes of traffic accidents [32]. Patients suffering from alcohol addiction, or any addiction to illegal drugs, have been found to have an increased tendency to use benzodiazepines [37]. Concurrent use of illegal drugs and/or alcohol can therefore be a plausible confounder in our study.

There was no information as to whether the drivers involved in the traffic accidents, as observed in this study, actually had consumed the prescribed medication before driving. Furthermore, if a hypnotic drug was in fact taken, there was no information on the time interval between the drug intake and the accident; the previous making it impossible to conclude on whether the hypnotic drug in question was actually present in the body at the time of the accident or not. The traffic accident may just as well have occurred due to a confounding factor related to being in a state of sleep medication requirement, i.e. any type of psychological imbalance, grief or recent undergone trauma. Insomnia, by itself, may just as well increase the risk of traffic accident involvement. The use of a prescribed, psychoactive drug as a confounder was, however, less likely: excluding all the individuals receiving other types of psychoactive drugs in addition to the hypnotic drug in question, did not alter the results.

Zopiclone is often prescribed for a longer duration of treatment, beyond the recommended 2 - 4 weeks [35]. The share of long-term users, among those involved in traffic accidents, was not known. In addition, the dose and the number of tablets prescribed were not considered.

There was no information on culpability, meaning that the setup did not differ between drivers ascribed to be the cause of the accident, and drivers through no fault of their own involved in an accident. Accordingly, an unknown share of the accidents included was obviously caused by other factors than the driver him-/herself (or his-/her prescribed medication).

The increased traffic accident risk, observed among the group of young male hypnotic users, could indicate stronger impairing effects by the use of hypnotics in this population group, as compared with females and older patients suffering from insomnia. However, the previous statement seems unlikely, there being no proof of a stronger drug effect on

younger male adults. Among the elderly, an increased drug effect has been reported, possibly due to the pharmacokinetic changes consistent with increasing age [48]. The increased traffic accident risk among the younger male drivers is more likely connected to a problem of selection: younger male drivers are overrepresented in the group of sleep related car crashes [104], and a concurrent use of alcohol and/or illegal drugs is proven to be a strong confounder for younger males [105]. Likewise, a study on drivers, aged 65 – 84, found that alcohol was not as likely a confounder among this group of elder drivers [33]. Altogether, the previous indicating a selection bias, related to a subpopulation of younger male drivers being at a particularly high traffic accident risk, as a result of younger males having personality characteristics leading to increased risk-taking behavior, a lower level of alertness, and/or concomitant drug use.

It seems likely that older people would tend to drive less than younger people; mileage was, however, not considered in this study. A higher traffic accident risk was found for the younger age group, in addition to a higher risk among males compared with females. The same trends were repeated for all of the hypnotics studied. Given that the presented trends continue beyond the age of 70, and this age group being included in the study, then the overall SIR, for traffic accidents related to an exposure to zopiclone, would have been somewhat lower. The previous did not, however, have an implication for the stratified results related to age groups in the presented study. Because people above the age of 70 were not included, the results should only be considered valid for the share of the population aged 18 - 69.

5.1.2 Paper II

The CTI is performed on individuals not necessarily representing the general Norwegian population of drivers. Because the study population to a large extent consist of drug addicts [24], this specific group of individuals may appear more “worn out” than others. The previous may, in turn, lead to a generally higher chance of being evaluated as impaired. It is widely known that zopiclone is one of the most commonly prescribed drugs among the elderly (and mostly among females), in Norway. Why then, does the study population of (mostly) apprehended drivers still consist of young male users of zopiclone? An explanation may be that the largest group of zopiclone users does not drive. Or if they do drive, they are not commonly apprehended by the police due to a “safer” appearance. It is likely that the police have a tendency to apprehend only the most impaired individuals. We have previously found a (slightly) positive relationship between blood amphetamine concentrations and impairment, as assessed by the (same) CTI [102]. The findings were followed by an academic discussion [106,107] . Seen in retrospect, the positive relationship, found for amphetamines, may have been revealed due to a selection of amphetamine-users on the descending limb of a binge abuse-period. The amphetamine concentrations may therefore have represented the length and intensity of the binge period, more than the effects of the amphetamine concentrations measured. Zopiclone differs greatly from amphetamines with regards to pharmacokinetics and pharmacodynamics, binge abuse is not reported, and one would expect an increasing concentration to represent greater impairment than a falling one. It is, however, of value

to acknowledge that the CTI results, in some way, probably may disclose more than the blood drug concentrations alone.

Based upon assumptions of a development of acute tolerance, an over-representation of individuals who have consumed zopiclone shortly before time of apprehension, is to be expected. Given the existence of a true positive concentration-effect relationship, the police will apprehend more individuals with high blood zopiclone concentrations. The study found very few moderate blood zopiclone concentrations, which supports the assumption of the mentioned selection bias; all together, implying that the share of impaired individuals in the included material, in general, is falsely elevated. Bachs et al. have previously documented that among apprehended drivers, with no positive findings in their blood samples, only 14 % were assessed as impaired [103]. As illustrated in Figure 6, the selection bias would be lower among the higher blood zopiclone concentrations, given a true positive concentration-effect relationship.

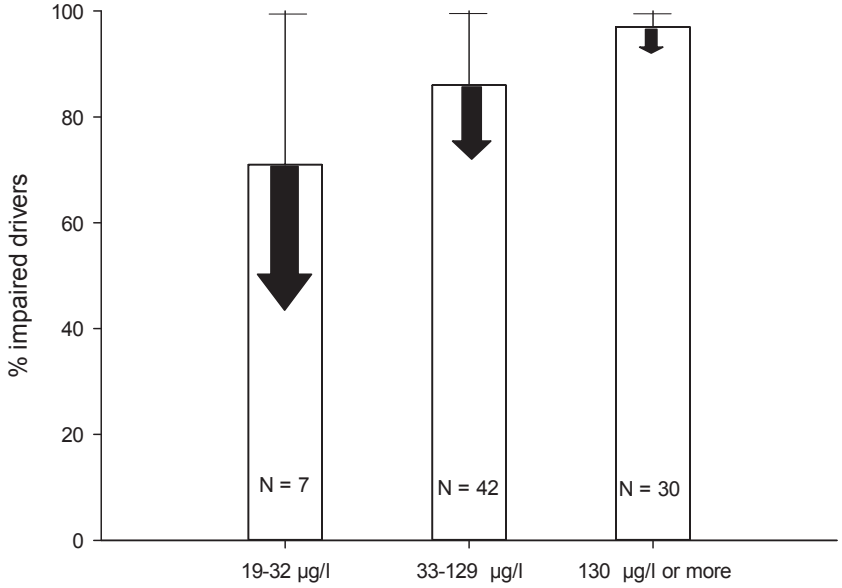


Figure 6: The share of apprehended individuals assessed as impaired, related to blood zopiclone concentrations in Paper II. The arrows indicating an assumption of true share of impaired observations without the selection bias

Recommendations have been made to investigate and document the reliability and validity of any test applied during experimental research [108]. Even though the setup in Paper II is observational (non-experimental), the reliability and validity of the CTI is relevant, as for any test used in an experimental study design.

Test Reliability for the CTI
The CTI inter-rater reliability is low due to different doctors performing the test under different conditions and circumstances. The test-retest reliability is not investigated.

Test Validity for the CTI
The validity of the Norwegian CTI is not documented.

There are no epidemiological studies on z-hypnotics evaluating traffic accident risk in relation to measured blood drug concentrations. In comparison, for ethanol the evidence is clear: The Long Beach/Fort Lauderdale study documents an exponentially increasing traffic accident risk related to BACs above 0.10 - 0.15 % [3]. To visualize the possible validity of the CTI results, the results from the Long Beach study are plotted together with the CTI results, calculated as relative risk (RR), in Figure 7. The figure illustrates that the RR for being assessed impaired did not change much with increasing BAC values. In contrast, the traffic accident risk increased considerably above the level of 0.10 %. Given that traffic accident is an applicable end-point, Figure 7 may point to a probable low CTI validity, and further accentuate the probable selection bias in Paper II among the most impaired drivers.

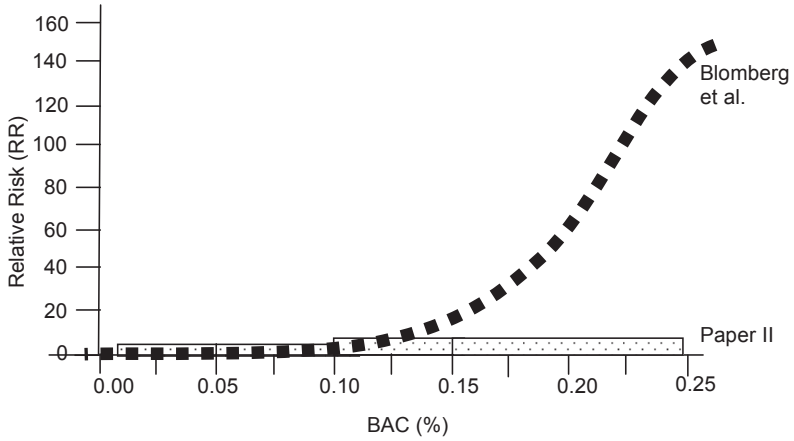


Figure 7: The predictive validity for the observations of impairment made in Paper II related to traffic accident risk for different BACs. The dotted line shows adjusted RRs for traffic accidents for BACs between 0.00 - 0.25 %, as documented by Blomberg et al. [3]. The bar graphs show RRs of impaired observations, as found in Paper II for BAC-positive cases (and in Bachs et al. for cases without any drugs present [103]): RR: 6, 6, 7 and 7, respectively, for the four BAC-groups shown

5.1.3 Paper III and IV

Healthy male volunteers were examined in the experimental study. This population group is not representative for the general Norwegian driver population, or for the population receiving zopiclone prescriptions, but the age and the gender are comparable to the population of suspected DUI drivers (Paper II).

The use of a crossover design lowers the chance of confounders, implying that the reported effects, for the most part, were caused by the actual drug effects. Still, other study populations (e.g. patients, older drivers or females) may have reacted differently to the tests due to inertly different pharmacokinetic- or pharmacodynamic responses. Because the psychomotor findings were related to measured blood drug concentrations, any possible pharmacokinetic differences among individuals are considered less relevant. A pharmacodynamic variation among different study populations has not been uncovered for zopiclone.

Three computerized tests were used to measure impairment: SOC, CRT and CPT. Different types of CRTs are commonly used in DUI research, while SOC and CPT are less often applied. Comparing results from different studies is difficult, due to different tests being utilized. A test battery, including tests for digital symbol substitution (DSST), memory, tracking and divided attention, has shown a low predictive validity for on-the-road SDLP performance [16]. SDLP is considered a reliable test for measuring traffic related impairment, and the low predictive validity indicated the mentioned tests to be less suitable. However, to measure SDLP is not necessarily the same as measuring traffic accident risk. There may be other aspects of behavior, necessary for safe driving, that are not covered by the SDLP test.

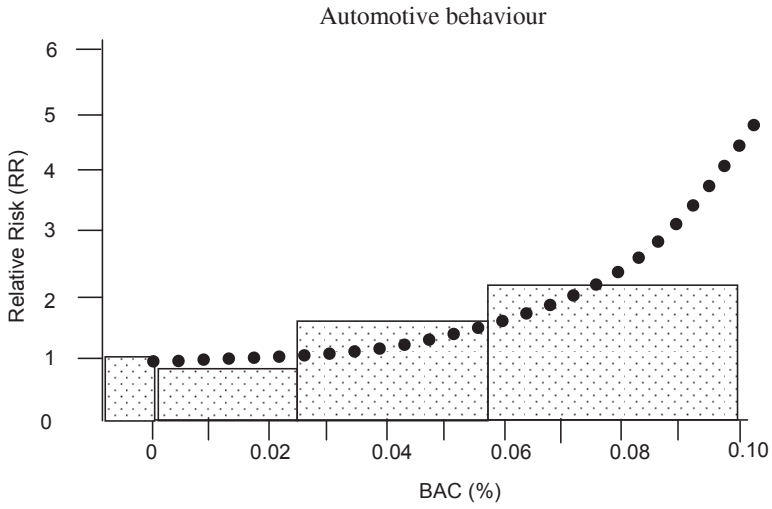
Test Reliability for SOC, CRT and CPT
Computerized tests were used, making the chance of inter-rater reliability high by definition.

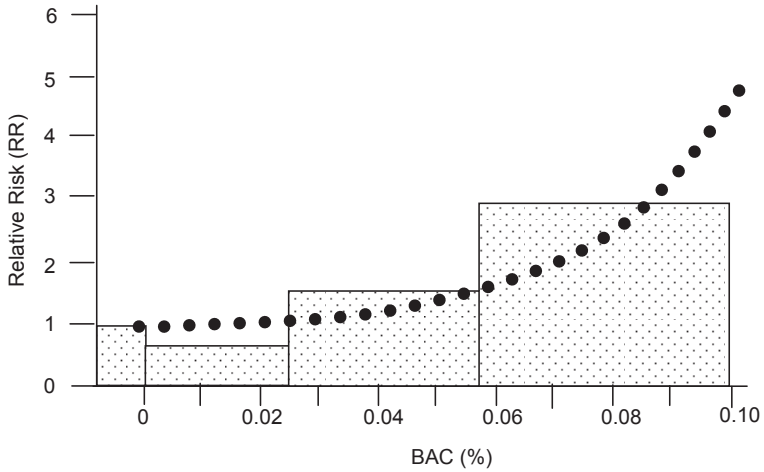
The test-retest reliability for the computerized test components was focused in different ways throughout Papers III and IV. In Paper III, the mean variance of the 4 baseline test values, for the 16 volunteers, are revealed in the appendix. However, the tables in the appendix do not explain whether the variance is related to intra- or inter-individual variance. A kind of test-retest reliability for each volunteer is considered in Paper IV, by using the study's dichotomized definition of impairment.

Test Validity for SOC, CRT and CPT
The test components included in the RCT, when regarded together, are meant to composite a picture of traffic relevant performance, based upon the three levels of behavior that are supposed to involve most aspects required for motor vehicle driving [8]. Given that the classification of the 15 test components into separate behavioural levels is correct, the content validity of the chosen test components, as a whole, can be considered quite high.

Traffic accident risk may be regarded as the end point, and therefore the most important aspect, in DUI research. As previously stated, the documented positive relationship between traffic accident risk and BACs, as documented by Blomberg et al.[3], may be considered the "gold standard", and is evidence of a true positive relationship between traffic-relevant impairment and BACs. The Long Beach/Fort Lauderdale study showed a (slightly) increasing traffic accident risk in relation to BACs between 0.00 - 0.10 [3]. In Figure 8, the definition of impairment, as applied in Paper IV, is used to visualize the

predictive validity of impairment in relation to the relative risk of a car crash. The figure reveals a higher predictive validity for the computerized tests, as compared with the CTI (showed in Figure 7).





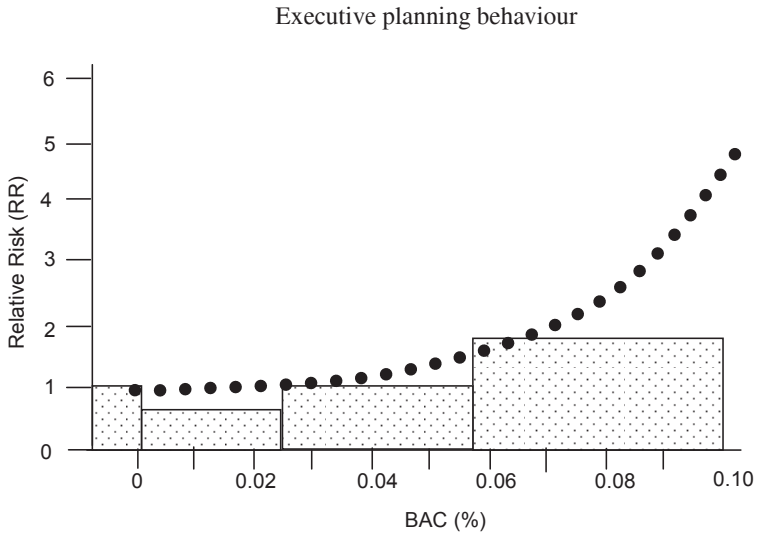


Figure 8: The predictive validity for the observations made in Paper IV related to traffic accident risk for different BACs. The dotted lines show adjusted RRs for BACs between 0.00 - 0.10 %, as documented by Blomberg et al. [3]. The bar graphs show RRs of impaired observations, as found in Paper IV. Results representing the three behaviour levels are shown separately

Post Hoc Power Calculation

A post hoc power calculation on the 15 included test components was attached to Paper III. A brief summary of the power results are given in table 8 below. It has been claimed that a post hoc power analysis only restates the statistical significance of the test, and that it will not add anything new of value [109]. On this basis, it should be underlined that the post hoc power analysis did not prove that the relationship between blood drug concentrations and impairment was underestimated.

Table 8: Results from the post hoc power analysis. The table shows the number of tests with sufficient power and, among them, the number of significant test results. Power is defined as 80 % and $P < 0.05$

		Zop 10	Zop 5	EtOH
Number of tests with sufficient power (N, %)	Automotive behaviour	6 (100 %)	5 (83 %)	2 (33 %)
	Control behaviour	2 (40 %)	0 (0 %)	2 (40 %)
	Executive planning	1 (25 %)	1 (25 %)	1 (25 %)
Number of significant tests results among the tests with sufficient power (N, %)	Automotive behaviour	5 (83 %)	2 (40 %)	1 (50 %)
	Control behaviour	2 (100 %)	0 (0 %)	2 (100 %)
	Executive planning	1 (100 %)	0 (0 %)	1 (100 %)

5.2 Aim 1: Traffic Accident Risk Related to Zopiclone Use

Before Paper I was conducted, only one epidemiological study had documented an increased traffic accident risk related to zopiclone exposure [9]. Our findings of increased traffic accident risk, observed during the first few days after exposure to a hypnotic drug,

is in accordance with previous traffic accident studies on both benzodiazepines [10,11,29-34] and the one mentioned regarding zopiclone [9]. Furthermore, the results here presented are in accordance with traffic accident studies carried out after the presented study, on both benzodiazepines [13,68,69,110,111] and z-hypnotics [13,68,69]. A low number of studies have found no significant traffic accident risk among users of benzodiazepines [112,113].

For all of these studies mentioned, confounding factors may have led to an overestimation of the traffic accident risk in relation to the drugs in question. Barbone et al. followed a case-crossover setup [9], which reduces the chance of confounding factors related to differences between individuals because the individuals are self-matched. Gjerde et al. calculated the risk of being involved in a traffic accident based upon analytical blood drug findings (where saliva was used for the controls), including both alcohol and illegal drugs, in a Norwegian case-control study [13]. The study found a significantly increased OR for fatally injured drivers for blood samples testing positive for zopiclone. The increased risk persisted in cases with blood samples testing positive for zopiclone alone (no other drugs or alcohol being present), indicating that confounding effects played only a minor role. In addition, a similar pattern was found for benzodiazepines in general, and for diazepam, except that there was no significant increased traffic accident risk for either benzodiazepines alone or for diazepam alone. Gjerde et al. found a higher OR for benzodiazepines and diazepam than for zopiclone, before excluding cases with more than one detected drug. These findings may underline the importance of confounding effects from other psychoactive drugs, even indicating that alcohol and illegal drugs are stronger confounders for benzodiazepines than for zopiclone. An interesting observation to be made is that zopiclone alone constituted an increased traffic accident risk, whereas benzodiazepines and diazepam alone did not. Unfortunately, the N was too low to study any of the specific hypnotic drugs other than zopiclone.

Another recent study aimed to exclude the use of alcohol as a confounder by requesting BAC analysis for all seriously injured drivers [68]. The study found a slightly increased risk of being responsible for the traffic accident with the use of zolpidem (OR = 1.29 (CI 1.09-1.52), and with the use of benzodiazepine hypnotics (OR = 1.39 (CI 1.08-1.79), but not with the use of zopiclone. The authors explain the difference between zopiclone and zolpidem as related to the patterns of drug use, or by factors personally related to the users. Usage trends may vary between countries. In Norway, people receiving benzodiazepine prescriptions are generally younger and more commonly male, as compared with those receiving prescriptions for z-hypnotics [35].There is no clear difference between those receiving zopiclone prescriptions as opposed to those receiving zolpidem, except for the group of patients receiving zolpidem being very small.

In addition, an epidemiological study, investigating culpability related to measured blood drug concentrations, found a clear, concentration-dependant relationship between alcohol and culpability [29]. This study also found a significant linear relationship for culpability, increasing with increasing blood benzodiazepine concentrations in combination with

other drug; likewise for benzodiazepines alone, indicating that benzodiazepines, by themselves, constitute an increased risk, with a positive concentration-effect relationship.

A lower SIR was observed for users of z-hypnotics compared with that of users of benzodiazepines. One should, however, be cautious when comparing the results of the different hypnotics with each other, due to the different material. Nevertheless, it is interesting to observe that the case-crossover calculations reduced the SIRs dramatically, for all of the investigated hypnotic drugs, leaving only significant SIRs for zopiclone and nitrazepam. In fact, the case-crossover results did, to some degree, confirm that there may have been factors, in connection to the users, even more relevant, to the cause of the increased traffic accident risk, than the effects of the drugs prescribed. Based upon a possible selection bias, one may argue that the increased traffic accident risk, found among the group of younger male drivers, probably has evolved due to the interaction of several factors: personality characteristics; confounding use of alcohol and/or illegal drugs; confounding factors related to being in a state of sleep medication requirement and sleepiness in general (which may to some extent be caused by the drug itself); as well as the specific drug impairing effects. The decreasing traffic accident risk, found among the higher age-groups, as well as for females, points to a possibly different weighting of the presented factors with increasing age and with the female gender.

Similar to benzodiazepines, z-hypnotics are not recommended for long-term use, and few studies have investigated tolerance development after longer usage. Based upon Neutel´s former observational studies on benzodiazepines, showing decreasing traffic accident risk, the longer the time had passed since collecting a benzodiazepine prescription [10,31], the higher a SIR was expected to be found among the incidental users, as compared to those exposed without a washout period. However, this was not found. In Paper I, similar SIRs were found after 14 days, as compared with 7 days after dispensing the hypnotics. The traffic accident risk after a 180-day washout was investigated for zopiclone only, due to the low number of cases involving zolpidem, nitrazepam or flunitrazepam alone. The difference lacking among the incidental users, as compared with those without a washout period, may be explained by a low level of tolerance developing to the impairing effects of zopiclone or it may be explained by a restrictive attitude towards driving a car related to patients receiving zopiclone rarely. Explaining the difference between the presented results and Neutel's results is difficult. Different patterns of confounding factors in Canada, as compared with Norway, may be one explanation. For example, a Finnish study concluded that current illness most probably played a larger role in explaining traffic accidents in Finland than in other countries [114], thus portraying how patterns influencing traffic accident risk can vary from place to place. The findings above may still serve as an argument for a low development of tolerance to the residual effects of the investigated sleep medications, thereby explaining how the increased traffic accident risk may persist throughout the exposure period. Based upon results in Paper I, it is, however, not possible to argue for an increased traffic accident risk persisting beyond 14 days of exposure.

Fatigue and a lack of sleep are both documented causes of road traffic accidents [104]. This documentation is based upon various methods, such as: surveys, police reports, and by collecting certain circumstantial evidence from the accident sites. The use of various

methods for documentation, makes the proportion of accidents, considered related to sleep deprivation, to show a great variation, ranging between 1 - 41% of all traffic accidents [104].

Sleep deprivation is difficult to relate to traffic accident risk, compared with drug intake or drug exposure. This is because sleep deprivation is an entity considered too diffuse to measure. Sleep disorders may, however, serve as indicators for sleep deprivation and sleep deficiency. Among the sleep disorders, the respiratory sleep disorders (like sleep apnea) have clearly been revealed as a risk factor for traffic accident involvement [104,115]. Among other sleep disorders, like insomnia, the evidence is not equally clear cut, but this may be due to lack of research in the field [115]. One may therefore question, based upon the previous, whether it is safer for a patient suffering from insomnia to avoid, or to consume, sleep medication, as prescribed by a physician, during the evening before daytime driving.

5.3 Aim 2: The Concentration-Effect Relationship between Zopiclone and Impairment

Paper II documents a positive relationship between high blood zopiclone concentrations and impairment, as assessed by the CTI. Seeing a positive relationship, despite the low CTI reliability and validity, as shown in section 5.1, may indicate that the observed positive concentration-effect relationship represents a robust phenomenon.

Papers III and IV documents a positive relationship, specifically for blood zopiclone concentrations ranging 16 – 74 µg/L, not differentiating between observations made shortly- or long after time of intake. Impairment was found for even lower blood zopiclone concentrations, during observations made less than an hour after intake. Papers III and IV reveal different responses at each behaviour level investigated, finding that automotive behaviour and control behaviour are more sensitive to the effects of zopiclone, in the range 0 – 74 µg/L, compared with that of executive planning.

Given a true positive concentration-effect relationship, a higher dose will lead to greater effects compared with that of a low dose. The previous statement is confirmed by the results in Paper III, by revealing greater impairing effects after consuming 10 mg of zopiclone compared with the effects after 5 mg, as documented for nearly all of the test components. Out of the 15 test components, only 4 demonstrate a significant difference between the two doses. A comparatively small difference between the doses, few observations, and relatively high intra- and inter-individual test variability may explain the lack of significant results. Among the 44 RCTs, as listed in the Appendix, 7 studies examined more than one zopiclone dose [60,61,70,76,116-118]; all demonstrating some level of a greater effect or a prolonged impairment by the higher dose.

Paper IV reports significant impairment above 16 µg/L for observations made at behavioural levels 1 and 2. Other studies have similarly found impairment below 20 µg/L, specifically for the tests: DSST, FFT and RT [79] at 3 - 6 h after intake, and for eye-hand coordination [76] at 10 h after intake. Like Kuitunen et al., Mattila et al. also

measured DSST at 1.5, 4.5 and 6 h after intake. Unlike the study performed by Kuitunen, Mattila found no significant DSST impairment at 4.5 h after intake, even though the measured mean blood zopiclone concentration was 45 µg/L [81]. Driving simulator studies have revealed diverging results, with respect to performance related to blood zopiclone concentrations: Bethelon et al. found no residual effects for 30 µg/L at 9 - 10 h after intake of 7.5 mg of zopiclone [75], as opposed to Bocca et al., who found residual impairment for 24 µg/L at 10 - 11 h after intake [80]. The two studies used different driving simulators and varying age groups of healthy volunteers, which may account for some of the differences.

Paper III documents impairment up until 3.5 h after intake. Other experimental studies have similarly revealed significant impairment up until 3 - 4 h after the intake of therapeutic sleep dosages (usually 7.5 mg of zopiclone) (63, 67, 68); while sensitive on-the-road studies have stated traffic relevant impairment as late as 10 - 12 hours after an intake of 7.5 mg of zopiclone [66,84,119], see Table 3 (Section 1.4.1.5). Paper IV reveals a development of acute tolerance for zopiclone, with blood drug concentrations below 16 µg/L showing a significantly higher share of impairment among observations made less than 1 h after intake, as compared with the observations made beyond 1 h. No other previous study has investigated acute tolerance for zopiclone in a similar way. One previous study aimed to investigate possible impairment following two different doses of zopiclone, and found evidence for acute tolerance: both a dosage of 3.75 mg and a dosage of 7.5 mg impaired the eye-hand-coordination, but the impairing effects following 7.5 mg of zopiclone lasted longer after time of intake (14 µg/L/10 h after intake) than that of 3.75 mg (20 µg/L/2 h after intake) [76].

Paper II is the first study documenting a positive concentration-effect relationship between blood zopiclone concentrations and CTI-results. Two former experimental studies, by Kuitunen et al., have tested a CTI on healthy volunteers after an intake of 7.5 mg of zopiclone [78,79]; both studies documenting significant impairment at 2 h after intake, but no significant impairment at 5 h after intake. The Kuitunen studies tested only one dose of zopiclone, and reported a mean blood zopiclone concentration at 1.5 h of 30 (+/- 5) µg/L and 37 (+/-10) µg/L, and at 5 h of 15 (+/-3) µg/L and 23 +/-3 µg/L. The lack of impairment at 5 h after intake may have been caused by the lower blood zopiclone concentrations and/or acute tolerance. The blood zopiclone concentrations in Paper II ranged between 23 - 1242 µg/L, with a median of 100 µg/L. Only 7 of the apprehended drivers in Paper II had a blood zopiclone concentration below 33 µg/L.

The measured blood zopiclone concentrations in Paper III and IV were lower than expected. As illustrated in Figure 3 (Section 1.4.1.6), this is in accordance with some of the former experimental studies. It should be underlined that Figure 3 only includes drug intake of 7.5 mg, and that even higher doses were given. The discrepancy between the studies may be related to one or more of the following factors: the zopiclone formulations, the study populations, the analytical procedures or the blood sampling procedures. Only one of the studies in Figure 3 used zopiclone tablets [79], while 7 studies used a capsular formulation, and one study failed to report the formulation [76]. Chromatographic methods were used to measure blood zopiclone concentrations in 6 of

the reported studies, and radioreceptor assay was used in one study [77] (the methods applied were not reported for 3 of the studies [75,76,81]). Finally, another possible explanation for the unexpected results may be the narrow time frames for the experimental setups. Because blood sampling and impairment testing both should take place within the short time frame of maximum impairment, most study designs will allow only one blood sampling within the first hour after drug intake. This sampling will therefore only on occasion coincide with the actual maximal blood zopiclone concentration. Taking several of these falsely low values and calculating the mean will give a misrepresenting result.

5.4 Aim 3: Impairment, Observed at Different Blood Zopiclone Concentrations, Expressed as BAC

In Papers II - IV we found a positive relationship between traffic-relevant impairment, for blood zopiclone concentrations, comparable to that of BACs, as shown in Figure 5 (Section 4.3). Papers III and IV both show that the effects of zopiclone and ethanol slightly differ with regards to the diverse test components. When the test components, at each behavioural level, are viewed as a whole, the effects of zopiclone and ethanol appear quite comparable, for each of the blood drug concentration levels tested.

Papers III and IV documents a somewhat differing impaired behaviour after the intake of zopiclone compared with that of ethanol. In short, zopiclone leads to a slower response, while ethanol increases the error tendency. Other studies on zopiclone or benzodiazepines versus ethanol have similarly found the drugs responding differently [78,120].

It is well known that impairment due to ethanol shows great inter- and intra-individual variation. The research, in the field of traffic-relevant impairment, is mostly based upon mean values for groups of individuals. One may ask if the results retrieved from the mean values are representative for a single individual. Especially with regards to tests with large inter-individual variation, mean results seem to be the least valuable. Based upon the same material, Paper III studies mean values, and individual values are studied in Paper IV. The results differ with regards to impairment at behavioral level 3, but in both papers the results are reported comparable for zopiclone and ethanol, at about the same blood drug concentration levels.

Paper IV reveals a relevant amount of acute tolerance developing, both for ethanol and for zopiclone. Former studies that have measured blood drug concentrations, often have not distinguished between results obtained shortly- or long after intake, meaning that the documented concentrations often are a mixture of rising- and decreasing values. However, most studies on the effects of zopiclone have related impairment in relation to time after intake. This may imply that the development of acute tolerance has been considered in practise. For alcohol, former studies have documented acute tolerance for e.g. speed and reaction time, but not for alcohol-increased errors [121]. “Errors” would most likely correspond to behavioural level 3. We saw no trends of less acute tolerance for behavioural level 3 compared to levels 1 and 2 when working with Paper IV.

Interestingly, acute tolerance for ethanol was not found in the DRUID meta-analysis [4]. In our Paper IV we distinguished carefully between absorption and elimination phase for each of the individuals. All the 16 volunteers were in the absorption phase (for ethanol as well as for zopiclone) at the point-of-time for the first CPT test session. The meta-analysis could not distinguish between absorption and eliminations that accurately. Schnabel et al. explained that their observations defined as being in the absorption phase probably also included some observations being in the elimination phase [4], which could explain a falsely elevated number of impaired observations in the "elimination phase" for the meta-analysis, and thereby explain the lack of acute tolerance in the DRUID report.

Table 4 in Section 1.4.1.6 shows four studies relating zopiclone impairment to BAC values. Only the study by Kuitunen found results comparable to the results presented [23,78]. The measured blood zopiclone concentrations in Mattila's study showed very high blood zopiclone concentrations, and were therefore not in accordance with presented results. Mamelak et al. [71] and Vermeeren et al. [84] found a significant impairment comparable to > 0.03 %, as long as 9 - 11 h after intake of 7.5 mg zopiclone, neither corresponding with the presented results. The divergence between the mentioned studies illustrates the complexity in attempting to find accurate blood drug comparisons for zopiclone and ethanol.

SDLP has been documented to reveal an unambiguous relationship between deviation and BACs [84,119,122-125]. A deviation of 2.5 cm has been found to represent an impairment level of 0.05 % [122], and 5 cm has been found to represent an impairment level at around 0.10 % [124]. Given that zopiclone induces a similar traffic-impairing effect, one could expect a steadily increasing relationship between SDLP findings and blood drug concentration for zopiclone. A certain blood zopiclone concentration could therefore be compared to a certain BAC level, by obtaining the measured deviation in the SDLP test. The measured SDLP deviation, 9 - 10 h after an intake of 7.5 mg of zopiclone, has been documented at 2 - 5 cm more than for that of placebo, and is comparable to BACs ranging 0.05 - 0.10 % [84,119,122-125]. On the one hand, the previous may be regarded as evidence of comparable effects of ethanol and zopiclone. However, the BACs, found comparable to the residual zopiclone effects, show a relatively broad range of variation, indicating that it may be difficult to make precise and distinct comparisons between the effects of zopiclone and ethanol. Blood zopiclone concentrations were not measured in the mentioned SDLP studies, and may have differed among the individuals. It is worth to mention that the most sensitive tests, like SDLP, often have been used in experiments long after drug intake, and usually after a night of (drug-induced) sleep. The DRUID meta-analysis suggests a time-of-day effect (for nitrazepam), indicating that the drug-induced sleep may have caused other, and stronger, drug-impairing effects than the effects following daytime administration [86]. However, it seems more likely that the most sensitive tests have been used in experimental studies on residual effects, and that the less sensitive tests have been used in daytime experiments, investigating impairment shortly after intake.

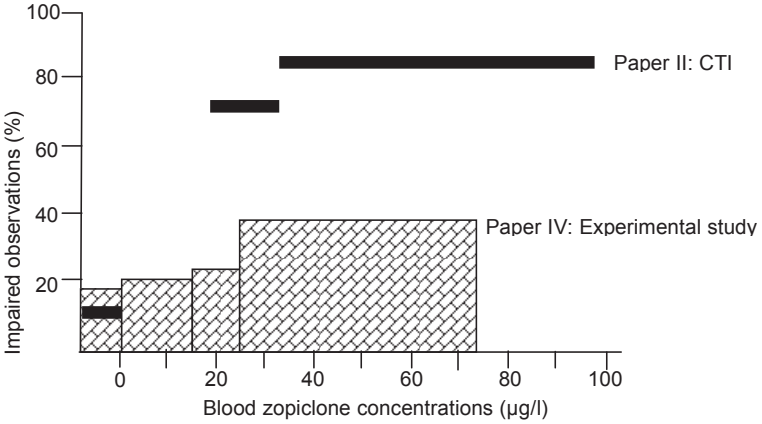


Figure 9: The percentage of impaired observations from two different study designs, related to blood zopiclone concentrations. The bar graph shows the percentage of impaired observations found in Paper IV, for all observations after zopiclone intake. The solid black lines represent the percentage of apprehended drivers, assessed as impaired by the CTI. The negative blood drug findings are from Bachs et al. [103], and the lines representing positive zopiclone findings are from Paper II.

As earlier mentioned, the DRUID meta-analysis compares a frequency of 50 % impaired observations to correspond with a BAC level of 0.08 %. Paper II reveals that even for the drivers with blood zopiclone concentrations between 19 - 32 µg/L, more than 70 % were assessed as impaired, and that nearly 100 % were impaired by the highest blood zopiclone concentrations. For ethanol, nearly 80 % were considered impaired by the lowest BACs (up to 0.05 %). These findings illustrate that the included material, in Paper II, was already selected (by the police) due to suspicious driving or due to traffic accident involvement. A material of randomly selected subjects, revealing the same blood drug concentrations as those included in Paper II, would probably have given a lower percentage of drivers being assessed as impaired. Figures 9 and 10 illustrate the divergence between the share of impaired assessments in Paper II and IV. It is interesting to observe that for zopiclone, as well as for ethanol, the RCT did not reach a level of 50 % impairment, even for the highest blood drug concentrations, when all behavioral levels were studied together. It should, however, be underlined that more than 60 % of the observations were evaluated as impaired, in Paper IV, among observations performed shortly after intake (see Figure 4 (acute tolerance) in Paper IV), both for zopiclone and for ethanol. One may therefore ask if the apprehended drivers have such a high frequency of impairment due to a selection bias of being “worn-out” drivers, or due to a selection bias based upon pharmacological appearance: apprehension shortly after intake of zopiclone or ethanol (acute tolerance).

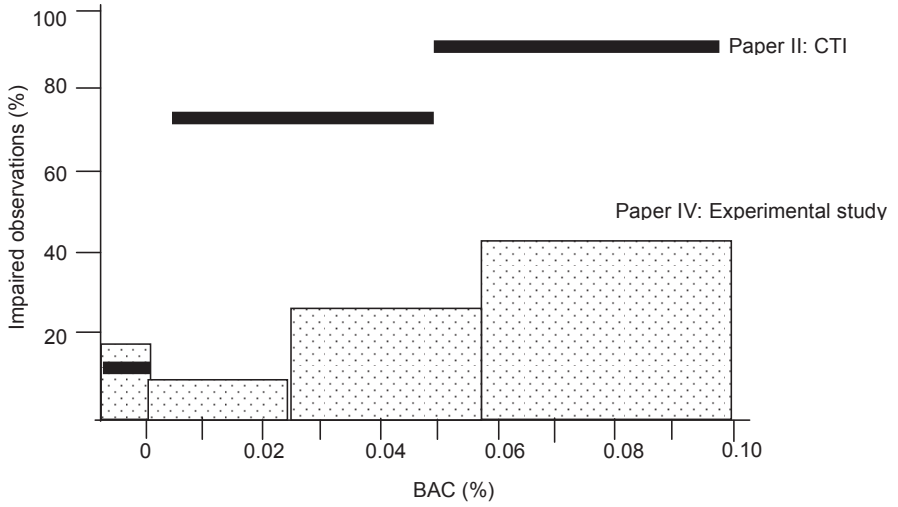


Figure 10: The percentage of impaired observations from two different study designs, related to BACs. The bar graph shows the percentage of impaired observations found in Paper IV, for all observations after ethanol intake. The solid black lines represent the percentage of apprehended drivers, assessed as impaired by the CTI. The negative blood drug findings are from Bachs et al. [103] and the lines representing positive BAC findings are from Paper II.

6. Conclusions

An increased risk of traffic accident involvement was found for drivers exposed to zopiclone. The risk was the highest for younger male drivers.

The increased traffic accident risk related to zopiclone exposure persisted throughout a case-crossover calculation, indicating a true drug-effect.

We found a positive relationship between blood zopiclone concentrations and impairment, starting at 16 µg/L. The positive relationship was sustained throughout the higher (supra-therapeutic) concentrations.

When studying the mean values, among the group of volunteers, impairment was not found beyond the first 3.5 hours after intake.

There is evidence of an acute tolerance development, both for ethanol and zopiclone, resulting in a decreasing level of impairment the longer the time after intake.

The concentration-effect relationships for zopiclone and ethanol were comparable to each other, within the blood drug concentration levels tested, except for that only zopiclone consumption gave a slow response and that ethanol consumption seemed to increase the chance of errors more than zopiclone.

Both zopiclone and ethanol showed some inter-individual variation with respect to impaired performance after intake. The variability for zopiclone did not differ from that of ethanol.

All in all, blood zopiclone concentrations seem as suited for traffic-related legal limits as BACs.

7. Suggestions for Further Research

Possible confounding factors, related to traffic accident risk, for users of zopiclone, need to be further explored. The previous can be examined by repeating the study while adding information regarding specific blood drug concentrations on the most commonly impairing drugs. Such a study could answer whether the drivers involved in traffic accidents actually consume their prescribed medication before driving, and which blood drug concentrations that are present at the time of an accident. Having a large N, such a study may answer some questions related to the significance of age and gender. If culpability was to be added, a more complete picture on traffic accident risk, related to the sleep medication, would appear.

More knowledge should be obtained about the extent of zopiclone abuse, e.g. the use of illegally obtained zopiclone and/or the use of supra-therapeutic doses. Because zopiclone is very commonly prescribed, it is important to reveal any knowledge on possible abuse potential. Such knowledge could be achieved by performing surveys. Roadside testing of human fluid (e.g. blood, urine or oral fluid) could (also) be performed to identify the extent of zopiclone abuse, in relation to traffic accident risk.

Tolerance needs also to be thoroughly investigated for zopiclone. If longtime usage leaves the user less impaired than single intakes, and if time after intake is a better predictor than blood drug concentrations, it may have large implications for interpreting results in forensic cases. RCTs on patients and healthy volunteers may be a suitable study design to explore the question of tolerance. Such a RCT on zopiclone patients is planned as part of an upcoming PhD study at the NIPH. In general, similar RCTs would be meaningful in forensic toxicology, and in clinical pharmacology, not only for zopiclone, but also for other common drugs of abuse (e.g. amphetamines, cannabis etc.).

8. Epilogue: The Present Handling of DUID cases in Norway

The legislation for 20 non-alcoholic drugs, in Norway, was altered to fixed blood drug concentration limits as of February 1st 2012 [126]. The introduction of the new system was made to harmonize the handling of all DUI cases, thereby decreasing the need for individual expert statements in cases with impairment due to non-alcoholic drugs.

Before introducing the system of legal limits, a workgroup, appointed by the Ministry of Transport and Communications, proposed blood drug concentration limits for 20 non-alcoholic drugs. For each of the 20 non-alcoholic drugs, 3 separate limits were set: a lower impairment limit, comparable to BAC of 0.02 %; and impairment limits for graded sanctions, comparable to BAC of 0.05 % and 0.12 %. Any blood drug concentration finding above the lower limit, in relation to operating a motor vehicle, is considered illegal; meaning that sanctions are required, unless the driver is able to provide evidence for the analytical findings being due to a prescribed treatment. Graded sanctions, such as conditioned imprisonment penalties, are to be sentenced if the driver's blood drug concentration corresponds to BAC above 0.05 %. If a blood drug concentration is higher than the corresponding BAC of 0.12 %, a sentence including unconditioned imprisonment is set.

As of February 1st 2012, a positive test result for any of the 20 psychoactive drugs will result in a request to the driver, where the driver is to produce evidence of a valid prescription for the drug in question. If the driver is unable to do so, then the driver will be sentenced by the fixed limits legislation system. If the driver is able to produce a valid prescription, an individual expert statement will be made.

The 20 non-alcoholic drugs were chosen by the workgroup due to certain specific criteria: they needed to have a potential for abuse, and/or they needed to constitute an increased traffic accident risk. The legislative limits were proposed based upon scientific knowledge, with studies on healthy volunteers.

Zopiclone is one of the 20 non-alcoholic drugs, as stated by the legislation of legal limits for traffic-relevant impairment. For zopiclone, the lower limit is 12 µg/L, the impairment limit comparable to a BAC of 0.05 % is 23 µg/L, and the impairment limit comparable to a BAC of 0.12 % is 58 µg/L.

9. Errata

Paper II:

- The cut-off value for the ethanol confirmation analysis is referred to in the text (under Methods) as 0.04 g/dL (%). The correct cut-off value should have been 0.004 g/dL (%).
- Analyzing Z-hypnotics were not implemented in the NIPH routine before July 2001. In Paper II it is referred to a screening of all samples from December 2000.

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11. Appendix

11.1 Overview of Relevant Experimental Literature

A MEDLINE-, EMBASE- and Pubmed-search was performed, with the following limits: English language, humans, not review or meta-study, objective test(s) on psychomotor impairment (not only subjective assessments), effects for zopiclone alone has been tested, the given zopiclone dose + time from intake to test is reported *or* blood drug concentrations at the time of testing has been measured.

Reference + Study design	**Study population**	**End points: (objective psychomotor measurements)**	**Setup**	**Exposure**	**Control**	**Results related to time after zopiclone intake (and/or zopiclone concentrations)**	**Comments**
[74] Allain et al 1995 Randomized double blind crossover	N=16 ♂ Healthy volunteers 23 +/-2 years	Attention / vigilance (CFF, stabilometric platform) and memory (letter recall test, recognition test and divided attention)	Single dose. Study medicine at 9:30 pm. Tests and blood sampling between 8:30 pm and 8:00 am	7.5 mg zopiclone, 10 mg zolpidem, 1 mg flunitrazepam, placebo (capsules)	Placebo	Zopiclone impaired attention and vigilance 50 minutes until 7 h after intake (25 µg/l). Zopiclone impaired memory not longer than 4 h after intake (40 µg/l).	Blood drug concentrations measured. Mean concentrations read from curve.
[127] Allain H et al 2003 Randomized	N=48 (49) Healthy volunteers	Attention / body sway (clinical	Single doses. Study medicines at	3.75 mg zopiclone, 5 mg zolpidem, 1	Placebo and baseline	Compared to placebo: Impaired body	Recall of 2-5 numbers digits were not impaired

double blind crossover	♀ and ♂ >65 years	stabilometric test, Simple RT, critical tracking test (CTT)) and memory (letter recall test, recognition test)	11:00 pm. Tests between 4-10 h after intake	mg lorametazepam, placebo (capsules)		sway until 8 h after intake. Increased reaction time until 9 h after intake	by zopiclone, only 6 digit numbers
[128] Berthelon et al 2003 Balanced randomized crossover	N=10 Healthy volunteers ♀ and ♂ 23-42 years	Driving simulator (errors, response time, distance estimation)	Single doses. Study medicines before bed at 11:00 pm. Tests at 9:00 am (10 h)	7.5 mg zopiclone, 10 mg zolpidem, 1 mg flunitrazepam, placebo (capsules)	Placebo	No residual impairment found 10 h after zopiclone intake (and also not after the other drugs taken)	Probably low test sensitivity due to lack of flunitrazepam residual effects
[75] Berthelon et al 2008 Balanced double blind crossover	N=16 Healthy volunteers ♀ and ♂ 20-40 years	Visual performance tested in driving simulator (collision anticipation and speed perception)	Single doses. Study medicines before bed at 11:00 pm. Blood sampling at 8:15 am. Tests at 9:00 am (10 h)	7.5 mg zopiclone, 10 mg zolpidem, 1 mg flunitrazepam, placebo (capsules)	Placebo	No residual impairment found at 30.3+/-15..9 µg/l (10 h) after zopiclone intake (and also not after the other drugs taken)	
[76] Billiard et al 1987 Latin-square double blind crossover	N=6 Healthy volunteers ♀ and ♂ 20-39 years	Eye-hand coordination test, CRT, DSST, word recall test	Single doses. Night 1 without medicines, night 2 bedtime intake. 3 test sessions	3.75 or 7.5 mg zopiclone, placebo	Placebo	Impaired eye-hand coordination >14 µg/l (2 h after 3.75 mg and 2 + 10 h after 7.5 mg), impaired CRT	

			8:00-12:00 am. Blood sampling.			>38 µg/l (2 h after 7.5 mg) No impairment >10 h	
[129] Bocca et al 1999 Balanced double blind crossover	N=16 Healthy volunteers ♀ and ♂ 20-30 years	Driving simulator (90 min test. Instructed to ensure lateral stability and to drive as quick as possible) + ocular saccade	Single doses. Bedtime intake at 11:00 pm. Tests performed 9:00 am or 11:00 am	7.5 mg zopiclone, 10 mg zolpidem, 1 mg flunitrazepam placebo (capsules)	Placebo	Impaired SDLP and ocular saccade at 9:00 am (10 h). No residual effects at 11:00 am	
[80] Bocca et al 2011 Double blind crossover	N=16 Healthy volunteers ♀ and ♂ 55-65 years	Driving simulator (60 min daytime monotonous driving): SDLP, speed deviation, driving off the road.	Single doses. Given at 11:00 pm Tests performed at 9:00 am the following morning. Blood sampling.	7.5 mg zopiclone, 10 mg zolpidem, 1 mg flunitrazepam, placebo (capsules)	Placebo	Residual effects all parameters (SDLP, speed deviation, driving off the road) 10 h after intake. Mean [zop] 25 µg/l	No correlation between driving parameter changes and measured blood zopiclone concentrations. More impairment for zopiclone and zolpidem compared to flunitrazepam
[61] Broadhurst et al 1987 Balanced randomized double blind crossover	N=10 Healthy volunteers ♀ and ♂ 28.2+/-4.9 years	Complex reaction time	Single doses. Bedtime intake. Tests 12 h after intake	2.5, 5, 7.5 and 10 mg zopiclone, placebo	Placebo	No impairment except 12 h after intake of 10 mg	
[70] Dehlin et al 1983	N=68 (75) Insomnia	DSST, letter cancellation test	Repeated doses.	3.75, 5, 7..5 or 10 mg	Baseline or placebo	No residual impairment	

Randomized double blind	patients, ♀ and ♂ 68-94 years		Bedtime intake. Screening + baseline 4 days, placebo 14 days, active treatment 14 days, placebo 7 days. Tests performed every morning	zopiclone		found	
[130] Farber et al 2008 Randomized double blind crossover	2 groups of healthy ♀ and ♂ volunteers: N=35 adults (18-45 years) and N=36 elderly (65-80 years)	DSST, SCT (symbol copying test),	Single doses. Adults: Bedtime 11:00pm, drug intake 03:00a m. Tests 4 and 6 h post dose. Elderly: Bedtime 10:00pm drug intake 02:00 are Tests 4, 6 and 8 h post dose.	Adults: 7.5 mg zopiclone, 10 and 20 mg indiplon, 10 mg zolpidem, placebo Elderly: 3,75 mg zopiclone, 5 and 10mg indiplon, placebo	Placebo	Adults: No sign effects DSST or SCT. Elderly: Zopiclone impaired DSST 4 and 8 h after intake	
[131] Fossen et al 1983 A) and B): Randomized	A) N=12 Healthy volunteers ♀ and ♂ mean age 25 years	A) Memory (retention, paired associates, visual memory test)	Repeated doses. A) Bedtime intake. Placebo day 1-7, active drug	A) 7.5 mg zopiclone, 2 mg flunitrazepam, placebo (capsules)	A) Placebo	A) Zopiclone impaired memory after 1st administration day.	

double blind crossover	B) N= 15 Healthy volunteers ♀ and ♂ mean age 23 years	B) Memory (digit learning, memory for position (remember where to place a symbol), visual memory)	day 8-14, placebo day 15-21, active drug day 22-28. Tests 11 h after intake days 1, 7, 8, 14, 15, 21, 22 and 28 B) Intake at 9:00pm. 4x3 days of active drugs separated by placebo washouts. Tests 10 h after intake day 1 and 3 in each period.	B) 7.5 mg zopiclone, 5 mg nitrazepam, 2 mg flunitrazepam, placebo (capsules)	B) Placebo	B) No residual impairment found	
[132] Griffiths et al 1986 Randomized double blind crossover	N=10 Healthy ♂ volunteers 20-22 years	Stroop test (e.g. "green" printed in red), Serial RT, letter recall, logical reasoning, memory	Single doses Intake 9:30am. Tests at baseline and 1, 4 and 10 h after intake.	7.5 mg zopiclone, 15 mg flurazepam, 1 mg lormetazepam, 0.25 mg triazolam, placebo (capsules)	Placebo	Zopiclone impaired SRT at 1 h (errors and latency), and increased mean time to complete logical reasoning until 4h.	
[133] Grobler et al 2000 Double blind	N=12 Healthy athletes	Eye-hand coordination, sprint-test,	Single doses. Intake at 10:00pm.	7.5 mg Zopiclone, 2 mg loprazolam,	Placebo	Did not impair any of the tests 10 h after intake	

crossover	♀ and ♂ mean age 22.8 (+/- 2.5)	graded treadmill	Tests performed next morning, 10 h after intake	placebo (capsules)		(same for loprazolam)	
[134] Harrison et al 1985 Randomized double blind crossover Same study as Subhan et al 1984 [135]	N=9 (10) Healthy ♀ volunteers 24-40 years	CFF, CRT, Sternberg test (information processing), tracking, mean brake time (simulated car driving)	Single doses. Evening intake. Tests before sleep (before drug intake and 1.5h after intake), and after sleep (10 h after intake)	7.5 mg zopiclone, 1 mg lormetazepam, 1 mg flunitrazepam, 0.25 mg triazolam, placebo (capsules)	Placebo	Zopiclone impaired only reaction time on the information processing task 1 h after intake. No zopiclone residual effects 10 h after intake	Only flunitrazepam had sign residual effects 10 h after intake
[136] Hemmeter et al 2000 Randomized double blind crossover	N=12 Healthy volunteers ♀ and ♂ (60-70 years)	CFF, CRT, letter cancellation test, memory (digit span / working memory and recall test / long term memory)	Single doses. Drug intake at 9:30pm, lights out at 10:00pm. Tests before intake (8pm), during sleep (2am) and after sleep (7am and 9am)	7.5 mg zopiclone, 20 mg temazepam, placebo (capsules)	Baseline	Zopiclone impaired CFF, CRT and simple attention as did placebo. For word recall only zopiclone impaired 4.5 h after intake (placebo NS)	
[137] Isawa et al 2000 Randomized double blind crossover	N=12 Healthy ♂ volunteers 28-42 years	Memory tests (word recall, passage recall and Sternberg test)	Single dose. Drug intake 8:00pm, bedtime 11:00pm, forced	7.5 mg zopiclone, 10 mg zolpidem , placebo (coloured tablets)	Placebo	No residual effects on memory 12.5 h after intake	

			awakened at 1:00am and 7:00am. Tests performed 1.5 and 12.5 h after intake				
[79] Kuitunen et al 1990 Randomized double blind crossover	N=12 Healthy volunteers ♀ and ♂ 22-35 years	Driving simulator (tracking, errors, RT), Divided attention, DSST, Maddox wing test, Flicker fusion, Body sway, CTI	Single doses. Tests performed + blood samples at baseline, and 1.5, 3, 4.5 and 6h after intake. CTI 2 and 5 h after intake	7.5 mg zopiclone, 600 mg carbamazepine; alone or in combination, placebo (tablets)	Baseline (placebo for CTI)	Zopiclone impaired tracking errors 1.5 h after intake (30+/-5 µg/l), CTI 2 h after intake, FFT and body sway until 3 h after intake (18+/-3 µg/l), RT until 4.5 h after intake (15 +/-3 µg/l) and DSST until 6 h after intake (12 +/-4 µg/l).	No clear effect of zopiclone on divided attention test.
[78] Kuitunen et al 1990 Randomized double blind crossover (Results from the study also published in 1994 [23])	N=12 Healthy volunteers ♀ and ♂ 20-28 years	DSST, Symbol copying, CFF, Maddox wing, Body balance, Divided attention, Simulator (5 min tracking and errors), CTI	Single doses. Tests performed + blood sampled at baseline and 1.5, 3, 4.5, 6 and 8 h after intake.	7.5 mg zopiclone, 0.25 mg triazolam, placebo (capsules) 0.8 g/kg ethanol, placebo (drink). Sleep medicines	Placebo (or baseline)	Zopiclone impaired tracking until 3 h, prolonged RT for 4.5 h (23 µg/l) and reduced DSST and symbol copying for 3 h after intake.	Cognitive tests and tracking most impaired, Slightly impaired RT, attention and tracking errors. Baseline values “fairly stable” (improvements in some tests)

				given alone or in combination with alcohol.			
[60] Lader et al 1983 Randomized double blind	N=10 Healthy ♂ volunteers 22-45 years	RT, tapping rate, DSST, Symbol copying test	Single doses. Bedtime intake. Tests performed 10 and 13 h after overnight intake	2.5, 5, 7.5 and 10 mg zopiclone, placebo (tablets)	Placebo	7.5 mg zopiclone impaired DSST until 13 h after intake and symbol copying test 10 h after intake. 10 mg zopiclone impaired tapping 10 h after intake and DSST, symbol copying and RT 13 h after intake	10 mg zopiclone did not impair RT 10 h after intake.
[119] Leufkens et al 2009 Randomized double blind crossover	N=18 Healthy volunteers ♀ and ♂ 55-75 years	Standardized highway driving test (SDLP, SDS) and laboratory tests (CTT, Divided attention, Stop signal, word learning, body sway)	Single doses. Bedtime intake awakened 8 h after intake. Driving test 10-11 h after intake and laboratory tests ca 12 h after intake	7.5 mg zopiclone, 20 mg temazepam, placebo (capsules)	Placebo	Zopiclone impaired SDLP 10h after intake, body sway 9 h after intake and impaired Stop signal task (inhibitory control), delayed word recall, impaired word recognition and delayed reaction time 12 h after intake	A similar SDLP impairment level (0.5 cm larger) has previously been found for BACs of 0.05%

[122] Leufkens et al 2009 Double blind crossover	N=28 (25) ♀ and ♂ Healthy volunteers 22-44 years	Standardized highway driving test (SDLP), and laboratory tests (CTT, Divided attention, DSST, word learning test, body sway)	Single doses. Zopiclone intake at 23:00 Lab tests 07:30-08:15 driving 09:00-10:00	7.5 mg zopiclone, 15 mg gaboxadol, 10 mg zolpidem, placebo (capsules)	Placebo	Zopiclone impaired driving 10-11 h after intake. Zopiclone impaired DSST, word learning and body sway 8.5-9 h after intake	A similar SDLP impairment level has previously been found for BACs of 0.05%
[71] Mamelak et al 1987 Randomized double blind	N=30 chronic insomnia patients ♀ and ♂ 32-60 years	DSST, immediate and delayed memory, backward masking, balance, critical tracking,	Multiple doses. Sleep medicine given at 11:00 pm 12 consecutive days. Tests performed first and last days of treatment, 11, 13.5 and 15 h after intake. (Alcohol intake at daytime, after sleeping)	7.5 mg zopiclone, 30 mg flurazepam, placebo (capsules) (0,5g/kg ethanol) Sleep medicines given alone or in combination with alcohol	Baseline and placebo	No residual effects for zopiclone	
[138] Mattila et al 1992 Double blind	N=18 Students	DSST, FFT, Maddox wing and driving simulator (2 min tracking test)	Single doses. Tests performed 0, 0.5 and 1.5 h after intake	7.5 mg zopiclone, placebo (uncoated tablets): with or without 300 mg caffeine /	Placebo	Zopiclone impaired DSST and Maddox wing, until 90 minutes after intake and FFT until 30 minutes	Only minor differences between zopiclone alone and zopiclone+caffeine

				decaffeinated coffee as placebo		after intake	
[81] Mattila et al 1994 Double blind crossover	N=12 Healthy volunteers ♀ and ♂ 19-32 years	"Global perfoamnce" (DSST, tracking errors and RT), body balance, simulated driving memory (recall)	Single doses. Daytime intake. Tests performed 0, 1.5, 3.5 and 6 h after intake. Blood sampling after each test session.	7.5 mg zopiclone, 0.4 mg suriclone, 50 mg chlorpromazine placebo (capsules)	Placebo	Zopiclone impaired "global performance" at 1.5 h (52 µg/l) and DSST until 3.5 h (45 µg/l).	
[77] Mattila et al 1997 Randomized, double blind crossover	N=12 Healthy volunteers ♀ and ♂ 19-30 years	Yes/No digit symbol substitution test (YNDST), symbol digit substitution test (SDST), digit-digit copying test (DDCT)	Single doses. Tests performed at baseline, 1, 3.5 and 5 h after intake. Blood sampling after each test session, expressed as diazepam equivalents.	7.5 mg zopiclone, 15 mg zolpidem, 15 mg diazepam, 30 mg oxazepam, placebo (capsules) 0.65+ 0.35 g/kg ethanol, placebo (drink)	Placebo: Delta performance (treatment – baseline) for active drug compared to delta performance for placebo	Zopiclone impaired YNDST and SDST 1 and 3.5 h after intake. No impairment 5 h after intake	Comparable results for zopiclone and ethanol for all time points, i.e. 0.082 % corresponded to 1 h after intake, 0.088 % corresponded to 3.5 h after intake and 0.060 % corresponded to 5 h after intake
[85] Mattila et al 1998 Double blind crossover (ethanol	N=12 Healthy volunteers ♀ and ♂ 21-28 years	DSST, Driving simulator (tracking, RT), body balance, CFF, memory (word recall	Single doses. Tests performed at baseline, 1, 3.5 and 5 h after intake.	7.5 mg zopiclone, 15 mg zolpidem, 15 mg diazepam, 30 mg	Placebo	Zopiclone impaired DSST, tracking error; RT and body sway 1 h after intake and	

results from Mattila et al 1997)		test)	Measured blood drug concentrations and BACs.	oxazepam , placebo (capsules) 0.65+ 0.35 g/kg ethanol, resp. placebo		DSST 3.5 h after intake.	
[139] Meskali et al 2009 Balanced double blind crossover	N=16 Healthy volunteers ♀ and ♂ 55-65 years	Driving simulator: (7 min urban driving performance 50 km/h, 5 accidents scenarios)	Single doses. Intake 11:00 pm. Test after sleep, 10 h after intake.	7.5 mg zopiclone, 1 mg flunitrazepam, 10 mg zolpidem, placebo (capsules)	Placebo	No significant impairment 10 h after intake (also not for flunitrazepam or zolpidem)	
[125] Mets et al 2011 Randomized double blind crossover	N=30 Healthy volunteers ♀ and ♂ mean age 25.9 (SD 6.5)	Standardized highway driving performance (SDLP, SDS), balance test (body sway), Psychometric tests (Word learning test, Sternberg test, Tracking test and Divided attention)	Single doses. Balance test at baseline. Intake 30 min before lights out. Balance test 1.5 h after intake, wakeup 7.5 h after intake. Lab tests and driving tests started 8.5 or 10 h after intake	7.5mg zopiclone, 8 mg ramelton, placebo	Placebo	Zopiclone impaired tests 8.5-10 h after intake: SDLP, reaction time, DSST, word delayed recall tracking. Zopiclone impaired balance at 1.5 h after intake.	A similar SDLP impairment level has previously been found for BACs of 0.05 %
[116] Mizuki et al 1983 Double blind crossover	N=16 ♂ Healthy university students	Arithmetic addition test	Single doses. Tests performed at baseline and 1	5 and 10 mg zopiclone, 5 mg diazepam, placebo	Placebo	Zopiclone impaired the test results in a dose-related	Zopiclone 5 mg impaired more than 5 mg diazepam

	20-25 years		h after intake	(tablets)		manner 1 h after intake.	
[140] Moon et al 1990 Randomized double blind crossover	N=12 ♂ shiftworkers 18-35 years	CFF, CRT, DSST	Multiple doses. Treatment 4 consecutive days at bedtime. Tests performed beginning and end of every shift.	7.5 mg zopiclone, placebo	Placebo	No impairing effects from zopiclone	Positive learning effect from first to second test cycle (Study performed by Rhõne-Poulenc ltd)
[118] Nicholson et al 1983 Double blind crossover	N=6 Healthy ♂ volunteers 21-33 years	DSST, symbol copy test	Single doses. Bedtime intake. Tests performed 9 h after intake.	2.5, 5, 7.5 and 10 mg zopiclone, placebo (capsules)	Placebo	7.5 and 10 mg zopiclone impaired DSST 9 h after intake. 10 mg also impaired symbol copy test 9 h after intake.	
[117] Nicholson et al 1987 Double blind crossover	N=6 Healthy ♂ volunteers 45-52 years	DSST, Complex RT, Symbol copying	Single dose. Bedtime intake. Tests performed 9 h after intake	5, 7.5 and 10 mg zopiclone, 30 mg flurazepam, placebo	Placebo	No residual impairment 9 h after intake	

[141] Paul et al 2001 Double blind crossover	N=13 (14) Healthy ♂ aircrew employees 22-50 years	Serial RT, logical reasoning task, serial subtraction task,	Single doses. Bedtime (circadian) intake. Tests performed hourly 7-14 h after intake	7.5 mg zopiclone, 10 mg melatonin, placebo (capsules)	Placebo and baseline	No impact on performance measured 7-14 h after intake compared to placebo.	All tests impaired post sleep compared to baseline. This effect was unrelated to drug intake
[82] Paul et al 2003 Double blind crossover	N=23 Healthy volunteers ♀ and ♂ 21-53 years	Serial RT, logical reasoning, serial subtraction, multitasking (25 minutes totally)	Single doses. Drugs taken 8:00am. Tests performed at baseline, and 0.25, 1.25, 2.25, 3.25, 4.25, 5.25 and 6.25 h after intake.	7.5 mg zopiclone, 6 mg melatonin, 10 mg zaleplon, 15 mg temazepam, placebo (capsules)	Placebo	Zopiclone impaired Serial RT, logical reasoning and serial subtraction from 1.25 h onwards, while multitasking from 0.25 h onwards. Impairment lasted until 6.25 h for logical reasoning and serial subtraction, until 5.25 h for Serial RT and until 3.25 h for multitasking.	Blood sampling was performed after each test. Blood drug concentrations shown in figures. Zopiclone levels peaked 1.75 h after intake; approximately 50 µg/l

[72] Ponciano et al 1990 Double blind parallel group	N=24 (26) insomnia patients ♀ and ♂ 18-60 years	CFF, CRT, letter cancellation, Digit span	Repeated doses. Drugs taken 5 consecutive weeks at bedtime. Tests performed early morning day 7, 14 and 21.	7.5 mg zopiclone, 30 mg flurazepam, placebo (capsules)	Placebo	No residual impairment zopiclone	
[142] Ramaekers et al 2011 Randomized double blind crossover	N=32 Healthy volunteers ♀ and ♂ Mean age 33 (SD 9) years	Standardized highway driving (SDLP) and cognitive / psychomotor tests (word learning, stop signal, critical tracking task, divided attention)	Single zopiclone doses: Esmirtazapine / placebo days 1-7 or placebo days 1-6 + zopiclone day 7. Driving test performed ca 11 h after intake days 2 and 8 and cognitive tests ca 13 h after intake.	7.5 mg zopiclone, 1.5 and 4.5 mg esmirtazapine	Placebo	Zopiclone impaired SDLP 11 h after intake Cognitive and psychomotor tests: No zopiclone impairment 13 h after intake.	Equivalent SDLP impairment level has previously been found for BACs of 0.05 %
[143] Rettig et al 1990 double blind randomized	N=60 Elective surgery patients ♀ and ♂ 18-65 years	Maddox wing, P-deletion test (deleting as many “p”-s from a text as possible)	Singe doses. Intake at 22:00 night before operation. Tests performed before intake and at 7:00am	7.5 mg zopiclone, 1 mg lormetazepam, 15 mg midazolam (capsules)	Baseline	Zopiclone impaired Maddox wing but not the P-deletion test 9 h after intake.	

[83] Saano et al 1992 Randomized double blind crossover	N=12 Healthy volunteers ♀ and ♂ 23 +/-2 years	Symbol copy test, DSST, tapping rate test, RT, FFT	Single doses. Drug intake 9:00 am. Tests performed at baseline and 1, 6, 8, 12 and 24 h after intake.	7.5 mg zopiclone, 5 mg diazepam, 1 mg lorazepam, placebo (capsules).	Placebo + baseline	Zopiclone did not impair any tests compared to placebo, but impaired the tests 1 h after intake when compared to baseline.	Blood drug concentrations measured but not shown in the manuscript
[144] Seppala et al 1982 Randomized double blind crossover (each receiving 3 of the 6 drug combinations)	N=20 Healthy ♂ volunteers 20-25 years	Body sway, tracking, reactive skills, flicker recognition, time perception, grip and pedal test	Single doses. Capsules given at 11:00 pm, drink given 8:30 am the following day. Tests performed at baseline, 9.5, 10.5 and 11.5 h after of capsules	7.5 mg zopiclone, 2 mg flunitrazepam, placebo (capsules) + alcohol or placebo (drink) Sleep medicines given alone or in combination with alcohol	Placebo	No residual impairment found for zopiclone alone, and the combination zopiclone + ethanol did not differ from ethanol alone.	(Study supported by Rhõne-Poulenc Santé)
[73] Staner et al 2005 Randomized double blind crossover	N=23 Insomnia patients ♀ and ♂ mean age 38.8 (+/- 2.0) years	Driving simulator: (collisions, speed, speed deviation)	Repeated doses. Bedtime intake 7 consecutive days. Tests performed 9-11 h post dose days 2 and 8.	7.5 mg , 10 mg zolpidem, 1 mg lormetazepam, placebo (capsules)	Placebo	Zopiclone increased number of collisions 9-11 h after intake. No other sign psychomotor effects.	Cannot see if the number of collisions differed between day 2 and 8 after zopiclone intake. (Study supported by Sanofi Aventis)
[135] Subhan et al 1984	N=9 (10) healthy ♀	Memory (Sternberg),	Single doses. Bedtime	7.5 mg zopiclone, 1	Placebo	Zopiclone impaired RT 1 h	

Randomized double blind crossover Same study as Harrison et al [134]	volunteers 24-40 years	RT)	intake. Tests performed 1 and 10h after intake.	mg flunitrazepam, 0.25 mg triazolam, 1 mg lormetazepam, placebo (capsules)		after intake, but not 10 h after intake (as did flunitrazepam)	
[145] Tada et al 1994 Randomized double blind crossover	N=8 Healthy ♂ volunteers 25-37 years	Standing steadiness (measured by stabilometer / microcomputer)	Single doses. Tests performed 1 and 2 h after intake	7.5 mg zopiclone, 0.25 mg triazolam, 5 mg nitrazepam	Placebo	Zopiclone impaired standing steadiness 1 and 2 h after intake.	Zopiclone impaired body sway more than nitrazepam and less marked than triazolam
[146] Uchiumi et al 2000 Randomized double blind crossover	N=12 Healthy ♂ volunteers 36 +/-5 years	Body sway, Tapping test, letter cancellation task, CFF	Single doses. Drug intake 8:00 pm, tests until 11:00pm, then 2 h sleep, tests (5 h after drug intake), sleep until 7:00am. Tests performed until 23 h after intake.	7.5mg zopiclone, 10 mg zolpidem, placebo (coloured tablets)	Placebo	Zopiclone impaired tapping rate (only) 12.5 h after intake.	No significant impairment for zopiclone and also not for zolpidem on any other tests.
[123] Vermeeren et al 1998 Double blind crossover	N=28 (29) Healthy volunteers ♀ and ♂ 23-40 years	Laboratory tests (body sway, word recall, spatial memory, reasoning, semantic verification) and	Single doses. Bedtime or nightly intake (awakened after 5h sleep); again 3 h sleep before wakeup. Lab	7.5 mg zopiclone, 10 and 20 mg zaleplon, placebo (capsules)	Placebo	Zopiclone impaired memory, body sway and semantic verification 4 h after intake. and impaired SDLP,	SDLP 4 h after intake 8.3 cm comparable to BAC ca 0.13 % and SDLP 10-11 h after intake comparable to BAC ca 0.10 %.

		standardized highway driving test (SDLP)	tests ca 9 or 4h after intake and driving tests 10-11h or 5-6h after intake.			reasoning and word recall 9-11 h after intake	
[84] Vermeeren et al 2002 I Randomized single blind crossover II Randomized double blind crossover	N=30 Healthy volunteers ♀ and ♂ 21-45 years	Standardized highway driving test (SDLP, SDS), laboratory tests (word learning (recall, recognition), CTT, divided attention)	Single doses I Study medicine intake afternoon. Tests performed same day. II Bedtime intake. Tests performed 10 h after intake	I 0.36g/kg / 0.43 g/kg ethanol + adjustable doses up to ca 0.050 % during test period, placebo II 7.5 mg zopiclone, 10 mg zaleplon, placebo (capsules)	Placebo	Zopiclone impaired driving (SDLP) and laboratory tests (word learning, divided attention) 10-11 h after intake	Zopiclone impaired driving and memory 10-11 h after intake > BAC ~0.030 % Ethanol impaired CTT while zopiclone did not. Mean BAC declined from 0.040-0.031 % during lab tests, and from 0.037-0.024 % during driving tests
[147] Warot et al 1987 Double blind crossover	N=12 Healthy volunteers ♀ and ♂ 22-30 years	CFF, CRT, DSST, short-term memory (word recall test), long-term memory (picture free/recognition recall)	Single doses. Tests performed at baseline and 2 and 6 h after intake	7.5 mg zopiclone, 0.25 mg triazolam, placebo (capsules)	Placebo	Zopiclone impaired anterograde short and long-term memory, CFF, CRT and DSST 2h after intake.	No retrograde amnesia detected for both zopiclone and triazolam, and no impairment 6 h after intake.

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IV