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Toxicokinetics and Organ-Specific Toxicity

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1. Introduction

Toxicokinetics (TK) refers to the kinetics of absorption, distribution, metabolism, and elimination (ADME) processes where both first and zero order kinetics are expected and these processes can vary over a wide range of doses. The goal of TK and pharmacokinetic studies are similar, which is to define the ADME properties of a drug candidate (Dixit & Ward, 2007). Therefore, the wide range of studies to define these ADME properties (e.g., in vitro and in vivo metabolism, animal mass balance, and distribution studies) performed in the pharmacokinetic evaluation of the drug candidate can also serve to help guide the toxicokinetic evaluation of the same drug candidate with the knowledge that first and zero order kinetics might be expected in the ADME processes at the higher doses of this drug candidate in the safety studies.

Now it is widely accepted that toxic effects can be better extrapolated from animals to humans when these comparisons are based on TK instead of dose alone. For example, the safety margin that is based on the ratio of the animal exposure at no observed adverse effect level (NOAEL) to human exposure at the efficacious dose is a key predictor of human safety risk. To calculate this safety margin, the animal and human exposure is determined by analyzing drug and metabolites concentrations in plasma, which is the most practical and widely accepted way of assessing this risk (Dixit & Ward, 2007). However, most safety issues are not observed in the plasma but in the organs and/or tissues. Therefore, is sampling plasma a good measure of the safety margin for the risk assessment of safety?

Sampling plasma and extrapolating this exposure to organs or tissues assumes that 1) concentration of drug in plasma is in equilibrium with concentrations in tissues, 2) changes in plasma drug concentrations reflect changes in tissue drug concentrations over time, and 3) distribution of drug and its metabolites is not affected by polarized cells (e.g., drug transporters and enzymes) that protect a lot of these tissues. Drug transport into tissues may not be a passive process and may depend on drug transporters (Ward, 2008), thus these assumptions may result in an inaccurate assessment of target organ exposure to drug and metabolites. Even without a drug candidate being a substrate for a drug transporter, lysosomal trapping of weak bases (e.g., liver and lung) or accumulation in membranes (e.g., muscle) can occur that can give rise to preferential distribution of the drug and its metabolites (MacIntyre & Cutler, 1988). Therefore, plasma is sometimes not a good

surrogate for tissue levels of drug and its metabolites, especially for the assessment of risk for some types of organ-specific toxicity.

The following case examples will illustrate how focusing on drug and metabolites in these tissues (where toxicity is observed) instead of plasma increases understanding of the nature of the toxicity and in some cases allows the efficient identification of a backup drug that has markedly less potential to cause that specific organ toxicity under investigation. These case examples are categorized by the different organs where toxicity was investigated and are generated from the author's personal experience in the pharmaceutical industry.

2. Case example: Toxicokinetics and testicular toxicity

This case example (described below) will highlight 1) preferential distribution of parent and metabolites to tissue, 2) a predominant metabolite that is different in the tissue versus plasma, and 3) accumulation of parent and metabolite that occurs in tissue and not in plasma. Furthermore, the case example will highlight that focusing on tissue burden of the drug and its metabolites (and not plasma concentrations) may actually ensure that a backup does not produce the same toxicity.

2.1 Testicular toxicity in rat

In a 13-week rat safety study, testicular atrophy was observed in rats at all doses tested (10, 50, and 250 mg/kg/day); however, these findings were not observed in the 2-week study. At the dose of 250 mg/kg/day, testicular atrophy was observed in approximately 50% of all rats. At doses of 10 and 50 mg/kg/day, these findings were observed in only 10% of rats but responsibility of Drug A for this toxicity could not be discounted (i.e., unequivocal). Therefore, no NOAEL could be assigned in this study, which markedly complicated the further development of this drug candidate.

2.2 Role of toxicokinetics in rat testicular toxicity

From the rat quantitative whole body autoradiography (QWBA) study, preferential distribution of Drug A-derived radioactivity to the testes was observed; furthermore, this radioactivity was retained in the testes markedly longer compared to other tissues (Figure 1). Since distribution of radioactivity included both parent and its metabolites and the dose in the rat QWBA study was based on the lowest dose of the rat safety study (i.e., 10 mg/kg/day), a cold study was initiated where rats were dosed with a single oral dose of Drug A at 50 mg/kg (similar to the mid dose in the rat safety study). After this single oral dose, the plasma, testes, and epididymes of the rats were collected at different time points and analyzed for Drug A and its two known metabolites (M1 and M2). Interestingly, the predominant metabolite in plasma (i.e., M2) was not the predominant metabolite in testes. M1 preferentially distributed to the testes from plasma; whereas, M2 had limited distribution to this tissue (Table 1 and 2). Furthermore, the T_{max} of M1 was 48 hours in testes suggesting a large accumulation potential of this metabolite in testes compared to plasma. Indeed after a follow-up study for six months of repeated daily oral dosing, M1 accumulated approximately five-fold in the testes; whereas, the parent did not accumulate (Figure 2). Furthermore, parent and M1 did not accumulate in the plasma during the 6-month rat safety study (data not shown).

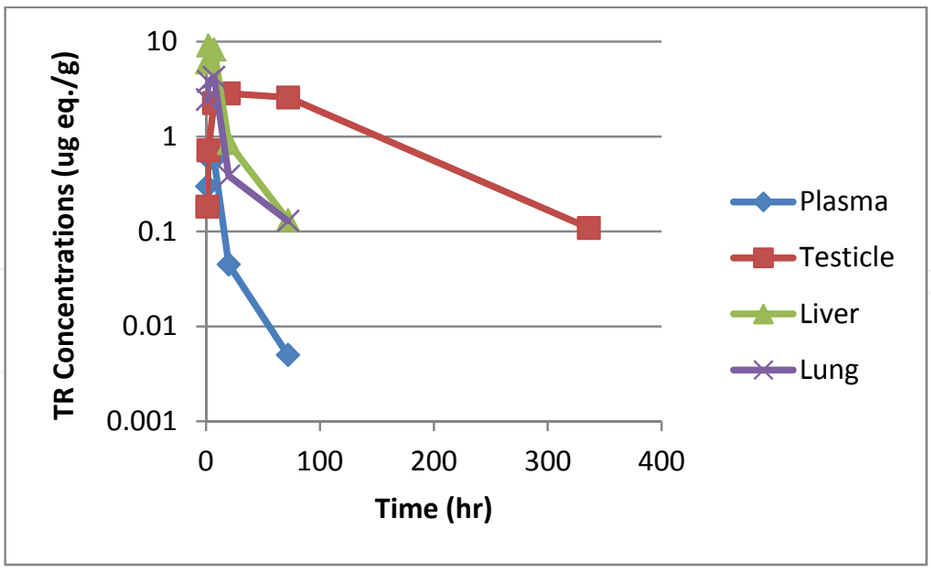

Fig. 1. Total Radioactivity (TR) Concentrations versus Time Profile of Drug A-derived Radioactivity in Rat Plasma, Testicle, Liver, and Lung.
Long Evans rats were dosed with a single oral dose of 10 mg/kg [^{14}C]-labeled Drug A. At different times after this dose, rats were sacrificed via exsanguination (cardiac puncture) under isoflurane anesthesia and blood (approximately 2 to 10 mL) was collected into tubes containing K_2EDTA immediately prior to collection of carcasses for QWBA. Samples were maintained on wet ice and refrigerated until aliquoted and centrifuged to obtain plasma. Immediately after blood collection the animals were prepared for QWBA. The carcasses were immediately frozen in a hexane/dry ice bath for approximately 8 minutes. Each carcass was drained, blotted dry, placed into an appropriately labelled bag, and placed on dry ice or stored at approximately -70°C for at least 2 hours. Each carcass was then stored at approximately -20°C. The frozen carcasses were embedded in chilled carboxymethylcellulose and frozen into blocks. Embedded carcasses were stored at approximately -20°C in preparation for autoradiographic analysis.

		Half Life (hr)	T_{max} (hr)	C_{max} (ng/mL or g)	AUC_{last} (ng*hr/mL or g)	AUC_{inf} (ng*hr/mL or g)
Plasma	M1	4	4	29	401	410
Plasma	M2	6	4	1033	11983	12025
Plasma	Parent	5	4	3712	43454	43491
Testes	M1	46	48	1182	156763	158157
Testes	M2	7	4	76	412	947
Testes	Parent	54	8	9061	684074	692652
Epididymes	M1	9	8	1441	25949	26064
Epididymes	M2	7	4	231	1215	2908
Epididymes	Parent	51	8	6676	115682	116647

Table 1. Toxicokinetic Profile of Drug A and its Metabolites in Rat Plasma, Testes, and Epididymes.

Fed Sprague Dawley rats (n=27) were administered a single oral dose of 50 mg/kg Drug A. Testes, epididymes, and plasma were collected at 1, 4, 8, 24, 48, 72, 96, 168, and 336 hours post dose from three rats at each time point. Bioanalysis of plasma, testes, and epididymes for Drug A (Parent) and its metabolites M1 and M2 was performed. Toxicokinetic parameters were determined on plasma, testes, and epididymes.

		C_{max}	AUC_{last}	AUC_{inf}
Testes	M1	40	391	386
Testes	M2	0.07	0.03	0.08
Testes	Parent	2	16	16
Epididymes	M1	49	65	64
Epididymes	M2	0.22	0.10	0.24
Epididymes	Parent	2	3	3

Table 2. Tissue to Plasma Ratios of Drug A and its metabolites in Rat Plasma, Testes, and Epididymes.

See description of Table 1 for experimental details. After toxicokinetic parameters were determined on testes, epididymes, and plasma, tissue to plasma ratios were calculated.

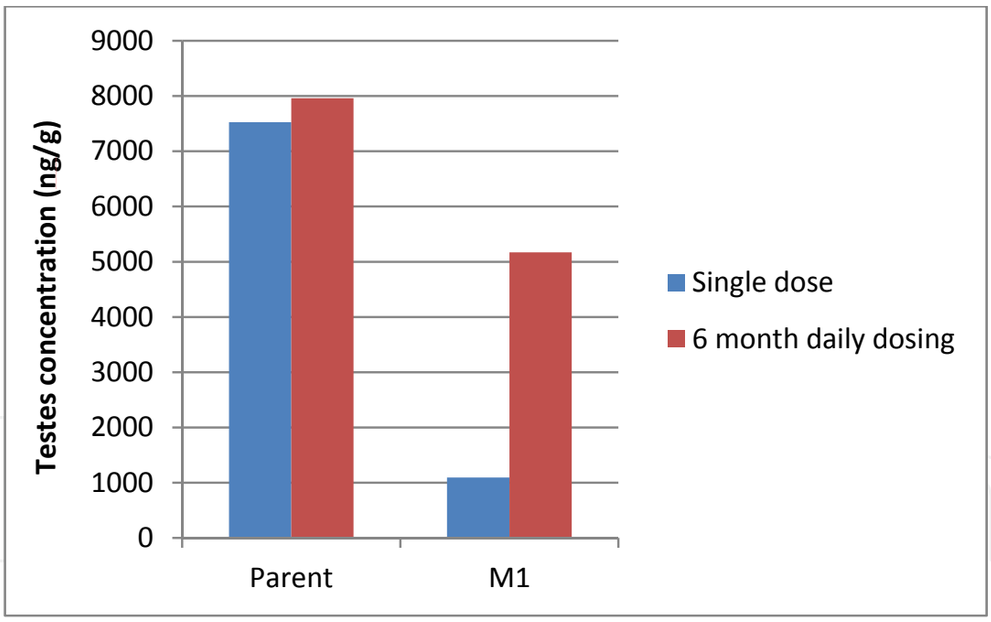

Fig. 2. Distribution of Drug A and its Metabolite, M1, in the Rat Testes after 6 Months of Repeated Daily Dosing (50 mg/kg/day) Compared to a Single Oral Dose (50 mg/kg)

Fed Sprague Dawley rats (n=4) were administered a single oral dose or repeated daily oral doses of 50 mg/kg/day Drug A for 6 months. Testes were collected at 24 hours post dose. Bioanalysis of testes for Drug A (Parent) and M1 was performed. Concentrations of Drug A and M1 after 6 months of repeated daily oral dosing (50 mg/kg/day) were compared to a single oral dose (50 mg/kg) at 24 hours post dose (see description of Table 1 for experimental details of the single oral dose study).

2.3 Identification of a backup molecule with limited potential for testicular toxicity

In order to identify a backup to this molecule (e.g., Drug A), screening potential backups in terms of their toxicity potential to rat testicular atrophy was not practical because of the time required for the toxicity to be observed (i.e., more than 2 weeks). Therefore, another method of screening potential backups needed to be initiated.

To aid the identification of this backup, the rat QWBA study of a prior drug candidate for this target (referred to as Drug B) was assessed where Drug B did not induce testes toxicity in rat during long-term safety studies. Interestingly, Drug B-derived radioactivity was approximately equivalent in blood and testes (Table 3), suggesting that the reduced burden of this tissue may have markedly lowered the susceptibility for this toxicity compared to the structurally similar molecule, Drug A. This markedly lowered distribution to the testes was also mirrored in the volume of distribution calculated after an single intravenous administration of Drug A and B, where the volume of distribution was markedly lower for Drug B compared to Drug A in every animal species tested (e.g., rat, dog, and monkey). Therefore to select future backups of Drug A into development, the volume of distribution was calculated from similar studies with administration of potential backup drug candidates via the intravenous route. These studies led to the identification of a potential drug candidate with similar distribution properties of Drug B (i.e, lower volume of distribution in every animal species tested after a single intravenous dose compared to Drug A). This potential backup to Drug A (referred to as Drug C) was then assessed in a rat QWBA study. In this study, Drug C-derived radioactivity was approximately equivalent in blood and testes (Table 4). From these encouraging results, Drug C was advanced into further development where no testicular toxicity has been observed in long-term rat safety studies. These results support the hypothesis that reduced tissue burden of the drug and its metabolites may actually predict that a backup does not produce the same toxicity.

Time (hr)	0.5	2	4	8	12	24	48	72	120
Blood	21.0	17.0	15.5	8.22	3.09	0.153	ND	ND	ND
Testis	2.84	7.54	15.4	9.98	5.26	0.346	0.135	BLQ	BLQ

Table 3. Tissue Concentrations (µg equivalents/g) of Drug B-derived Radioactivity in Rat Plasma and Testis.

Long Evans rats were dosed with single oral dose of 30 mg/kg [^{14}C]-labeled Drug B. See description of Figure 1 for experimental details.

Time (hr)	1	4	8	24	72	168	336
Blood	4530	1280	310	58.3	BLQ	ND	ND
Testis	2240	989	456	105	BLQ	BLQ	BLQ

Table 4. Tissue Concentrations (µg equivalents/g) of Drug C-derived Radioactivity in Rat Plasma and Testis.

Long Evans rats were dosed with a single oral dose of 10 mg/kg [^{14}C]-labeled Drug C. See description of Figure 1 for experimental details.

2.4 Conclusion

Knowledge of tissue toxicokinetics will increase the understanding about the potential mechanism of an organ-specific toxicity and can potentially assist in identifying a backup drug candidate that has a markedly lower potential for this organ-specific toxicity.

3. Case example: Toxicokinetics and liver toxicity

This case example (described below) will highlight an investigation into liver toxicity where the mechanism of the liver toxicity was questioned. This drug candidate induced a strong pharmacological response; therefore, an investigation was launched to investigate whether the liver toxicity induced by this drug was a result of its strong pharmacology or an off target effect (i.e., independent of its targeted receptor pharmacology) from one of the metabolites of the drug.

3.1 Liver toxicity in dog

In a dog toleration study at the lowest dose tested (10 mg/kg), slight, acute central-lobular and portal inflammation with individual hepatocyte necrosis was observed. Therefore, no NOAEL could be assigned in this study which markedly complicated the further development of this drug candidate.

3.2 Role of toxicokinetics in dog liver toxicity

Even though this drug candidate was known to elicit a strong pharmacological response that could be capable of inducing the adverse effect observed in the dog toleration study, the potential of this drug candidate to form an acyl glucuronide (M2) in liver was evident and thus this metabolite may also be the cause of these adverse effects (Kenny et al., 2005). Furthermore, the potential preferential distribution of this drug candidate to the liver may also predispose its adverse effects. Therefore to investigate these hypotheses, the plasma and liver (also kidney and fat for comparison) were analyzed for drug candidate and its metabolites in the dog after 14 days of repeated daily oral doses of the drug candidate (i.e., parent).

After toxicokinetic evaluation of the tissues and plasma, the concentrations of parent in liver were consistently lower than plasma at 2, 6, and 24 hours postdose, suggesting no preferential distribution of the drug to the liver (Table 5). Furthermore, the acyl glucuronide metabolite (M2) along with other metabolites (M1, M3, and M4) were only observed in the plasma and not in the liver (Table 6), suggesting that these metabolites were not the cause of the observed liver toxicity. These results suggested that the observed liver toxicity in dog was caused by the strong pharmacological response of the drug candidate and probably not caused by an off target effect of M2 (or any other metabolites observed in plasma). Furthermore, the lack of preferential distribution of parent to the liver indicated that the toxicokinetic analysis of plasma exposure was correct in evaluating the risk for observed liver toxicity in the potential further development of this drug candidate.

3.3 Conclusion

Toxicokinetic evaluation of tissue (where toxicity is observed) and plasma for drug and its metabolites will allow further mechanistic understanding of the cause of the observed tissue toxicity and will aid in the choice of the most relevant matrix for sampling in order for the correct evaluation of risk in further development of the drug candidate.

Day	Time (hr)	Concentration (μg/mL or g)							
		Plasma	Plasma + Acid	Liver	Liver + Acid	Kidney	Kidney + Acid	Fat	
Day 1	2	292	373	130	168	209	180	59	Mean
		35	114	17	24	46	68	11	SD
	6	286	284	131	96	181	123	65	Mean
		112	92	35	12	102	57	4	SD
	24	54	48	46	28	68	34	61	Mean
		44	37	24	16	37	25	5	SD
Day 14	2	295	381	226	140	245	90	73	Mean
		141	208	100	27	160	38	12	SD
	6	293	275	284	128	187	94	71	Mean
		89	110	72	29	27	35	5	SD
	24	39	37	41	29	52	36	72	Mean
		54	53	40	24	61	43	3	SD

Table 5. Concentration-Time Profile of Parent in Dog Plasma, Liver, Kidney, and Fat.

Fed Beagle dogs (n=18) were administered a single oral dose or repeated daily oral doses for 14 days of 10 mg/kg drug. Liver, kidney, fat, and plasma were collected at 2, 6, and 24 hours post dose from three dogs at each time point with and without formic acid (formic acid was added to potentially increase the stability of the acyl glucuronide metabolite). Bioanalysis of liver, kidney, fat, and plasma for drug candidate was performed.

Metabolite	Type	Plasma		Plasma + Acid		Liver		Liver + Acid	
		Day 1	Day 14	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
Parent	-	116,860,326	123,174,663	122,389,879	122,716,072	27,431,252	36,110,995	28,993,390	29,924,921
M1	Oxidation + Sulfation	ND	ND	ND	ND	ND	ND	ND	ND
M2	Glucuronidation	5,299,597	3,790,520	3,552,836	2,817,400	ND	ND	ND	ND
M3	Oxidation	ND	ND	ND	ND	ND	ND	ND	ND
M4	Oxidation	453,680	1,035,337	553,582	1,045,264	ND	ND	ND	ND

ND = not detected

Table 6. Peak Area Counts Versus Time Profile of Parent and its Metabolites in Dog Plasma, Liver, Kidney, and Fat.

Fed Beagle dogs (n=18) were administered a single oral dose or repeated daily oral doses for 14 days of 10 mg/kg drug. Liver, kidney, fat, and plasma were collected at 24 hours post dose from three dogs at each time point with and without formic acid (formic acid was added to potentially increase the stability of the acyl glucuronide metabolite). Bioanalysis of liver, kidney, fat, and plasma for drug (Parent) and it metabolites (M1, M2, M3, and M4) was performed. Peak areas were integrated for both parent and metabolites in each matrix. Data from kidney and fat are not shown.

4. Case example: Toxicokinetics and central nervous system (CNS) toxicity

This case example (described below) will highlight an investigation into CNS toxicity where the lead drug candidate displayed CNS toxicity in the monkey and a backup molecule needed to be identified. This example highlights utilization of the efflux transporter, P-glycoprotein (Pgp), to limit the tissue distribution of the backup drug candidate to the CNS in order to limit CNS toxicity potential.

4.1 CNS toxicity in monkey

In a Cynomolgus monkey toleration study at the 100 mg/kg/day dose (repeat daily oral dosing), test article-related clinical signs observed in the male monkey were characterized by vomiting, ptosis, decreased activity, prostration, tremors, convulsion and ataxia. A slight safety margin was identified (approximately 7-fold); however, this margin was not large enough to confidently advance this drug candidate into longer GLP safety studies in monkey.

4.2 Role of toxicokinetics in monkey CNS toxicity

Unfortunately, the brains of these monkeys were not sampled after the monkey toleration study. However, plasma and brain exposures in the mouse were known for this drug candidate. Mice express similar membrane proteins (e.g., Pgp and BCRP) in their blood brain barrier compared to Cynomolgus monkeys (Ito et al., 2011); therefore, we hypothesized that brain penetration of this drug candidate in mouse may approximate the respective brain penetration in monkey.

The brain to plasma ratio of this drug candidate was large (i.e., 22) in mouse; furthermore, drug was retained in the mouse brain compared to plasma (Figure 3). These results suggested that the drug candidate was preferentially distributed to the brain with a large accumulation potential. This large accumulation potential suggested that the safety margin (established in the monkey toleration study) might decrease with the increased duration of the safety studies, further compromising the developability of this lead candidate.

4.3 Identification of a backup molecule with limited potential for CNS toxicity

In order to identify a backup molecule with limited potential for the observed CNS toxicity of the lead drug candidate, screening potential backup molecules for CNS toxicity in monkey would be resource intensive. Furthermore from an animal usage and management perspective, reduction of potential primate mortality was optimal. Since toxicological screening for a potential backup was unfavorable, reduction in the distribution of a backup to the CNS was a possible solution. Marked structural alterations of the physiochemical

properties for this chemical series to alter CNS distribution were not possible since these alterations markedly reduced potency for the pharmacological receptor. Interestingly, some of these molecules (in the same chemical series) were identified as substrates for Pgp. In the MDR1-MDCK cell model, the efflux ratio of the Pgp substrates was between 2 and 3. Since Pgp is known to reduce CNS distribution through efflux of drug candidate from the apical membrane of the endothelial cells in the blood brain barrier into the blood (Cordon-Cardo et al., 1989), the effect of Pgp on the CNS distribution of these potential backup molecules was determined in the mouse (as discussed previously, monkeys were not a practical model for this exploration). CNS concentrations were approximately 10-fold less for one of these backup drug candidates compared to the lead drug candidate (Figure 4). Therefore, this backup drug candidate was advanced into clinical trials and CNS toxicity was never observed in monkey and human.

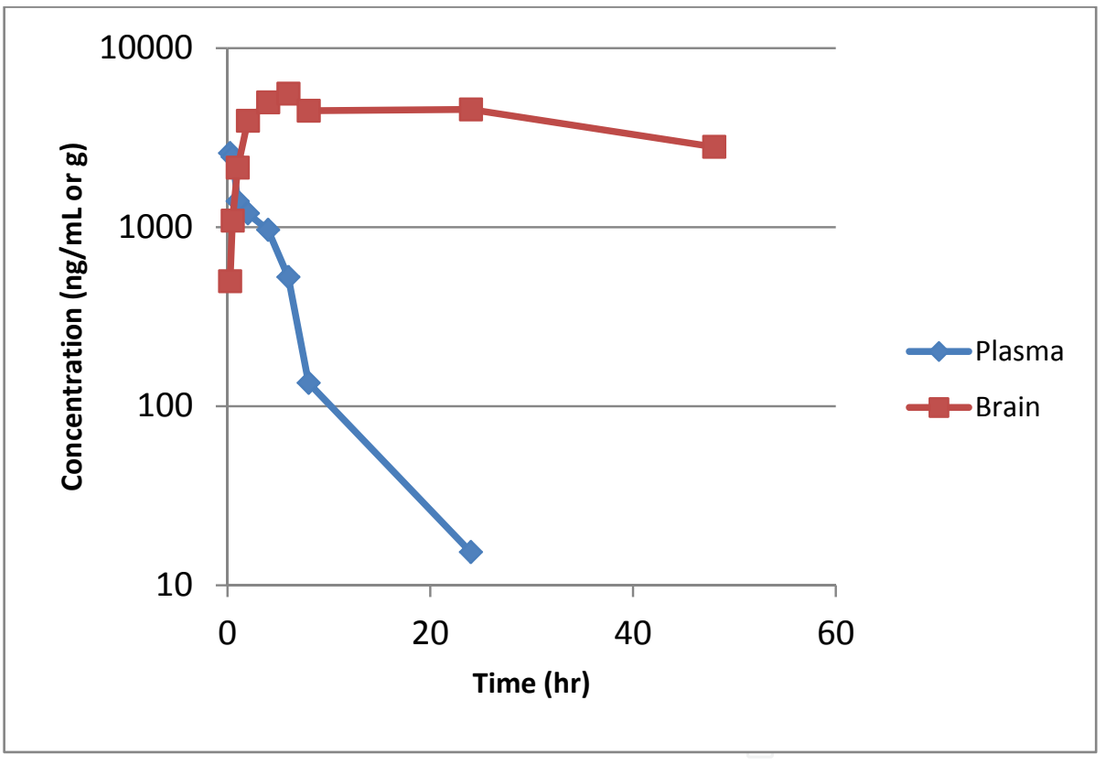

Fig. 3. Concentration-Time Profile of Lead Drug Candidate in Mouse Brain and Plasma after a Single Oral Dose (20 mg/kg)
Fasted CD1 mice (n=27) were administered a single oral dose (20 mg/kg) of the lead drug candidate. Brains and plasma were collected at 0.25, 0.5, 1, 2, 4, 6, 8, 24, and 48 hours post dose from three mice at each time point. Bioanalysis of brain and plasma of the lead drug candidate was performed.

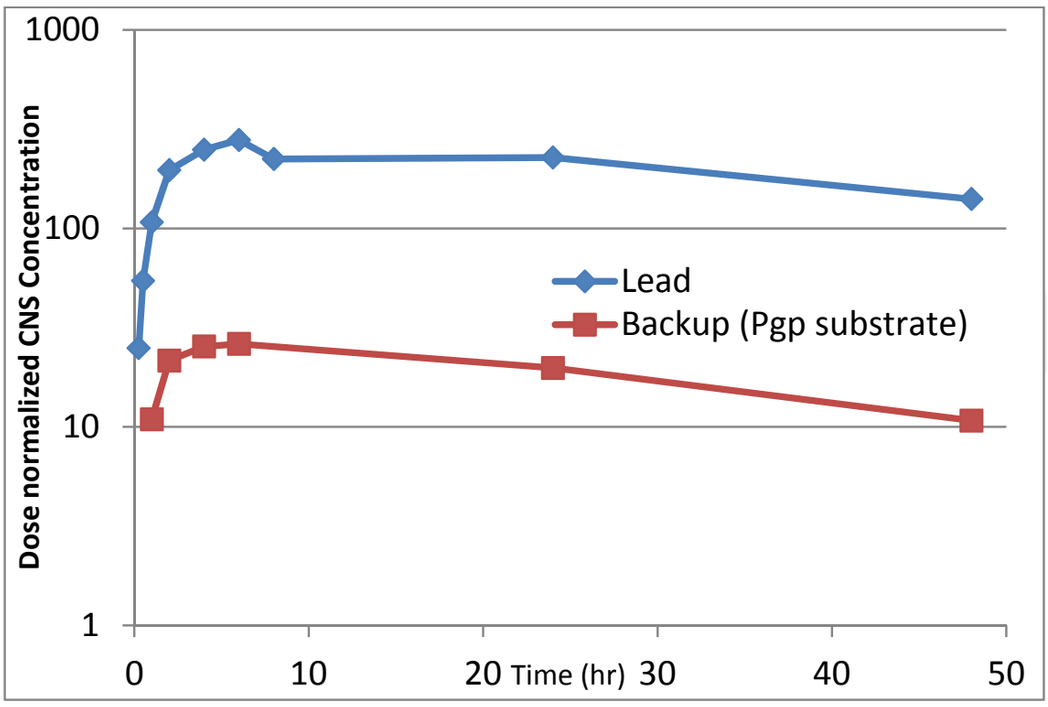

Fig. 4. Dose Normalized CNS Concentration-Time Profile of Drug Candidate in Mouse Brain and Plasma after a Single Oral Dose (20 mg/kg for lead and 5 mg/kg for backup)
Fasted CD1 mice (n=54) were administered a single oral dose of 20 mg/kg of the lead or 5 mg/kg of the backup drug candidate. For the lead drug candidate, brains were collected at 0.25, 0.5, 1, 2, 4, 6, 8, 24, and 48 hours post dose from three mice at each time point. For the backup drug candidate, brains were collected at 0.5, 1, 4, 6, 24, and 48 hours post dose from three mice at each time point. Bioanalysis of brains for the lead and backup drug candidate was performed.

4.4 Conclusion

Development of a backup drug candidate that is a substrate for efflux transporters which limit its distribution to the CNS (e.g., Pgp) can reduce the potential for this backup to cause CNS toxicity where the prior lead drug candidate demonstrated this toxicity in animal safety studies.

5. Future use of safety margins in tissues

In the future, more thorough risk assessment of safety will include safety margins from exposure of drug and its metabolites in the tissues (in addition to plasma) where organ specific toxicity is observed. The challenge in this endeavor is the assessment of drug exposure in human tissues since these tissues cannot be easily sampled from most human volunteers. Therefore, creative sampling methods must be applied. For example, noninvasive in vivo measurements such as sampling excreta (urine, feces, bile, and semen) will be useful. Furthermore, in vitro systems such as the hepatocyte sandwich-culture model (Chandra & Brouwer, 2004), and humanized mice (Jiang et al., 2011), combined with physiologically based pharmacokinetic (PBPK) modelling (Kusuhara & Sugiyama, 2010) can also replace the need for direct sampling of human tissues.

5.1 Sampling excreta to estimate drug and metabolites in tissues

The concept of sampling excreta to estimate drug and metabolites in human tissues is still evolving. The importance of understanding absolute abundance of metabolites from sampling excreta was highlighted by the need to understand the importance of metabolites in safety testing or MIST (Baillie et al., 2002; Smith & Obach, 2005). Smith and Obach concluded that the risk assessment of metabolites would seem more prudent if it was based on absolute mass and not proportion of drug-related material (Smith & Obach, 2005); therefore, sampling excreta and analyzing total amount of metabolite excreted would be more useful than sampling plasma (especially at higher dose of the drug). The recommendation for sampling excreta was based on determining the entire body burden of the metabolites for this MIST guidance and less about sampling excreta to estimate drug and metabolites in tissues.

In animals, the concept of sampling excreta to estimate drug and metabolites in tissues has been applied in a limited fashion. For example in beef steers treated with gentamicin, a small residue remains bound to the kidney cortex tissue for many months (this residue is unacceptable at the time of slaughter). Interestingly, plasma levels of gentamicin declined rapidly to no detectable levels within 3 days after intramuscular administration of gentamicin, while measurable amounts in urine persisted for 75 days before the concentration of gentamicin declined to levels too low to quantitate by the available liquid chromatography tandem mass spectrometry (LC/MS/MS) technique (Chiesa et al., 2006). An estimated correlation between an extrapolation of urine gentamicin concentration to the corresponding kidney tissue sample suggested a urine to kidney tissue relationship of 1:100. A test system sufficiently sensitive to a urine gentamicin concentration of 1 ng/mL correlated with the estimated 100 ng/g gentamicin limit applied to the fresh kidney of the recently slaughtered bovine (Chiesa et al., 2006). This example highlights the utility of measuring excreta (e.g., urine) to better estimate concentrations of drug in tissue (e.g., kidney).

The challenge of excreta being a surrogate model to assess concentrations of drug and metabolites in human tissues is the limited understanding of how concentrations of drug and metabolites in the excreta will relate to the concentrations in the respective tissue. This challenge can be minimized by establishing a relationship between the concentration of drug and metabolites for excreta and tissues in animals (as illustrated by the above example with gentamicin in beef steers). In addition, translating that relationship from animal to human with in silico tools (e.g., PBPK modelling) and in vitro and in vivo human models (e.g., primary in vitro human cell models and humanized mice) will increase the confidence in including safety margins from exposure of drug and its metabolites in the tissues (in addition to plasma) where organ specific toxicity is observed. Below is a case example where the utility of semen as a surrogate model to assess the concentrations of drug and metabolites in dog testes was investigated.

5.1.1 Case example: Utility of semen as a potential matrix to estimate drug and metabolites in testes

In this case example, the potential of semen was evaluated as a matrix to determine the concentration of Drug A (same drug candidate described in the section for Toxicokinetics and Testicular Toxicity) and its metabolite (M1) in dog testes (for potential extrapolation to human). For this study, dogs were given a single oral dose of Drug A and then at different

time points dogs were ejaculated to collect semen and their testes were sampled. The toxicokinetic profile of M1 in semen and testes was similar (Table 7). Furthermore, the exposure of parent in testes also approximated the exposure of parent in semen where the exposure of Drug A in semen was approximately 2.5-fold higher than the exposure in testes (Table 7). These results suggest that semen approximated the exposure of Drug A and M1 in testes. Therefore, excreta may be a possible surrogate matrix to estimate tissue concentrations of drug candidate and its metabolites; however, supplementary systems like primary in vitro human cell models and humanized mice, combined with PBPK modelling, will be needed to extrapolate these results to human.

		Half Life (hr)	T_{max} (hr)	C_{max} (ng/mL or g)	AUC_{last} (ng*hr/mL or g)	AUC_{inf} (ng*hr/mL or g)
Testes	M1	13	7	2890	80787	81831
Testes	M2	3	1	28	87	108
Testes	Parent	18	7	12000	232701	233251
Semen	M1	16	7	3680	75766	75867
Semen	M2	ND	ND	ND	ND	ND
Semen	Parent	5	4	42700	593334	593391

Table 7. Toxicokinetic Profile of Drug A and its Metabolites in Dog Testes and Semen.

Before dosing, Beagle dogs (n=9) were trained for ejaculation 2 times/week. Dogs were administered a single oral dose of 15 mg/kg Drug A. Testes (n = 1/time point) were collected at 1, 4, 7, 24, 48, 72, 96, 168, and 336 hours post dose from dogs at each time point. Semen was also collected in the period between dosing and sacrifice. Bioanalysis of semen and testes for Drug A and its metabolites, M1 and M2, was performed. Toxicokinetic parameters were then determined.

5.2 Utility of PBPK to estimate drug and metabolites in tissues

PBPK models aid in the understanding of the disposition of chemicals in the body in different animal species, including humans. In toxicological research, PBPK modelling was initiated approximately 30 years or so, and mainly from an environmental toxicology perspective. For example, PBPK models were developed for polychlorinated biphenyls, methylene chloride, and other persistent lipophilic compounds starting in the mid 1980s (Andersen, 1995). In the past, the utilization of PBPK models in safety assessment departments within the pharmaceutical industry was not common, although the utilization of PBPK models is gaining momentum.

The utility of PBPK models is to extrapolate from one environment to another; for example, PBPK models extrapolate from high to low dose, different routes of administration, interspecies, and different durations of exposure. All of these extrapolations are potentially needed to bridge knowledge of drug and metabolites concentrations in the tissues of safety assessment species (e.g., rat, dog, and monkey) to human tissues (Thompson et al., 2007).

For the best extrapolation, the mechanism of interaction leading to toxicity would be known; for example, a known biological process that is disturbed by a known entity, parent, and/or metabolite (Andersen, 1995). However in many cases, this mechanism is not known and PBPK models can assist in possibly identifying these mechanisms. Especially when modelling efforts address the appropriate questions, the systematic discovery of these mechanisms is possible. The key is to develop models with appropriate measures of tissue concentrations in animals and possibly excreta concentrations in animals and humans. To strengthen this extrapolation, in vitro systems, such as primary in vitro human cell models (e.g., hepatocyte sandwich-cultured cell model and proximal tubule cell monolayers), and humanized mice, will also provide vital parameters (e.g., pharmacokinetics rate constants) for the PBPK modelling in order to extrapolate tissues concentrations from animal to human. In 2001, a consensus building workshop sponsored by the Society of Toxicology concluded that the human in vitro systems, through quantitative measurements and PBPK modelling, can play an important role in dose-response assessment (MacGregor et al., 2001). Therefore in the near future, the combination of these technologies may allow researchers the ability to estimate drug and metabolites concentrations in human tissues.

5.3 Utility of supplementary human models to estimate drug and metabolites in tissues

The primary challenge in calculating safety margins in tissues where organ-specific toxicity is observed is the access to human tissue samples for the measurement of drug and its metabolites. One method to address this challenge is to simulate the distribution of drug and its metabolites in a human in vitro model. For example, development of valid and reliable techniques to quantify biliary excretion of drugs in healthy human volunteers is difficult. Measurements of drug concentrations in bile can only be obtained from patients diagnosed with diseases of the gallbladder and biliary tract who require medical procedures that allow this measurement (Ghibellini et al., 2006). However, there is a promising, recent technique to estimate bile in healthy human volunteers with an oroenteric catheter to aspirate duodenal secretions, and gamma scintigraphy to determine gallbladder contraction. This technique allowed the comparison of the biliary clearance of three compounds estimated with sandwich-cultured human hepatocytes (a human in vitro model). The rank order of biliary clearance predicted from in vitro corresponded well with the in vivo biliary clearance values in mL/min/kg for Tc-99m mebrofenin (7.44 vs 16.1), Tc-99m sestamibi (1.20 vs 5.51), and Tc-99m piperacillin (0.028 vs 0.032) (Ghibellini et al., 2007). Since sandwich-cultured human hepatocytes need to uptake drug across their sinusoidal membrane in order to excrete the drug across their canalicular membrane for the in vitro measurement of biliary excretion, this verification of a good prediction of this human in vitro model from the clinical study suggests that the intracellular concentration within these sandwich-cultured human hepatocytes can also estimate concentrations of drug in the human hepatocyte in vivo. Therefore, in vitro models have the potential to supplement costly and difficult sampling in healthy human volunteers to estimate drug and metabolite concentrations in tissues and excreta. However, significantly more research is needed to realize this potential in existing models and to expand the amount of models for in vitro human tissue models.

Another possible human model to estimate drug and metabolites in organs and/or tissues is mice with humanized organs and/or tissues. To create this model, a severe combined immunodeficient (SCID) mouse line is injected with human cells from the human tissue into

the respective mouse tissue. For example, injection of cryopreserved human hepatocytes through a small, left flank incision into the inferior splenic pole in a SCID mouse created a mouse with humanized liver that was replaced by more than 80% of human hepatocytes (Okumura et al., 2007). In this chimeric mice model, cefmetazole (CMZ) excretions in urine and feces were 81.0 and 5.9% of the dose, respectively; however, excretions in urine and feces in control SCID mice were 23.7 and 59.4% of the dose, respectively (Okumura et al., 2007). Because CMZ is mainly excreted in urine in humans, the excretory profile in chimeric mice was demonstrated to be similar to humans. Interestingly in the chimeric mice, the hepatic mRNA expression of human drug transporters (e.g., MDR1, BSEP, MRP2, BCRP, OCT1, and OATP1B1/1B3) were detectable; whereas, the hepatic mRNA expression of mouse drug transporters in the chimeric mice was significantly lower than in the control SCID mice (Okumura et al., 2007). In conclusion, chimeric mice exhibited a humanized profile of drug excretion, suggesting that this chimeric mouse line would be a useful animal model to predict human ADME. Most studies have focused on humanized liver models; however, the potential for humanization of other organs and/or tissues in the mouse is evident in the near future. These new potential models will markedly improve the ability to estimate drug and metabolite concentrations in human organs and/or tissues.

6. Conclusion

For the determination of a safety margin, drug and metabolites concentrations are sampled in plasma, which is the most practical and widely accepted way of assessing this risk. However, most safety issues are not observed in the plasma but in the organs and/or tissues. Assumptions about concentrations of drug and metabolites in tissues from extrapolation with plasma may result in an inaccurate assessment of target organ exposure to drug and metabolites. Therefore, plasma is sometimes not a good surrogate for tissue levels of drug and its metabolites, especially for the assessment of risk for some types of organ-specific toxicity.

Knowledge of toxicokinetics of an organ-specific toxicity can potentially assist in identifying a backup drug candidate that has a markedly lower potential for this organ-specific toxicity. Therefore, a hypothetical plan may be generated where focusing on tissue burden of the drug and its metabolites may actually ensure that a backup does not produce the same toxicity. For example, identifying a backup drug candidate with limited tissue distribution to the tissue where organ-specific toxicity was observed (e.g., testicular toxicity) markedly reduced the potential of these backups to cause these toxicities; furthermore, development of a backup drug candidate that is a substrate for efflux transporters which limit its distribution to the CNS (e.g., Pgp) can reduce the potential for this backup to cause CNS toxicity.

In the future, innovative models such as 1) noninvasive in vivo measurements such as sampling excreta (e.g., urine, feces, bile, and semen), 2) in vitro systems, such as primary in vitro human cell models (hepatocyte sandwich-cultured model), 3) humanized mice, and 4) PBPK models, will provide more insight into the concentration of drug and metabolites in human organs and/or tissues. Therefore, these innovations will provide a more thorough risk assessment of safety which will include safety margins from exposure of drug and its metabolites in the tissues (in addition to plasma) where organ specific toxicity is observed.

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8. References

Andersen, M.E. (1995). Development of physiologically based pharmacokinetic and physiologically based pharmacodynamic models for applications in toxicology and risk assessment. *Toxicol Lett,* Vol.79, No.1-3, pp. 35-44, ISSN 0378-4274

Baillie, T.A., Cayen, M.N., Fouda, H., Gerson, R.J., Green, J.D., Grossman, S.J., Klunk, L.J., LeBlanc, B., Perkins, D.G., & Shipley, L.A. (2002). Drug metabolites in safety testing. *Toxicol Appl Pharmacol,* Vol.182, No.3, pp. 188-196, ISSN 0041-008X

Chandra, P., & Brouwer, K.L. (2004). The complexities of hepatic drug transport: current knowledge and emerging concepts. *Pharm Res,* Vol.21, No.5, pp. 719-735, ISSN 0724-8741

Chiesa, O.A., von Bredow, J., Heller, D., Nochetto, C., Smith, M., Moulton, K., & Thomas, M. (2006). Use of tissue-fluid correlations to estimate gentamicin residues in kidney tissue of Holstein steers. *J Vet Pharmacol Ther,* Vol.29, No.2, pp. 99-106, ISSN 0140-7783

Cordon-Cardo, C., O'Brien, J.P., Casals, D., Rittman-Grauer, L., Biedler, J.L., Melamed, M.R., & Bertino, J.R. (1989). Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc Natl Acad Sci U S A,* Vol.86, No.2, pp. 695-698, ISSN 0027-8424

Dixit, R., & Ward, P.D. (2007). Use of Classical Pharmacokinetic Evaluations in Drug Development and Safety Assessment, In: *Toxicokinetics and Risk Assessment*, Lipscomb, J.C., & Ohanian, E.V. (Ed.), pp. 95-122, Informa Healthcare USA Inc., ISBN 978-0-8493-3722-2, New York, NY

Ghibellini, G., Leslie, E.M., & Brouwer, K.L. (2006). Methods to evaluate biliary excretion of drugs in humans: an updated review. *Mol Pharm,* Vol.3, No.3, pp. 198-211, ISSN 1543-8384

Ghibellini, G., Vasist, L.S., Leslie, E.M., Heizer, W.D., Kowalsky, R.J., Calvo, B.F., & Brouwer, K.L. (2007). In vitro-in vivo correlation of hepatobiliary drug clearance in humans. *Clin Pharmacol Ther,* Vol.81, No.3, pp. 406-413, ISSN 0009-9236

Ito, K., Uchida, Y., Ohtsuki, S., Aizawa, S., Kawakami, H., Katsukura, Y., Kamiie, J., & Terasaki, T. (2011). Quantitative membrane protein expression at the blood-brain barrier of adult and younger cynomolgus monkeys. *J Pharm Sci,* Vol.100, No.9, pp. 3939-3950, ISSN 1520-6017

Jiang, X.L., Gonzalez, F.J., & Yu, A.M. (2011). Drug-metabolizing enzyme, transporter, and nuclear receptor genetically modified mouse models. *Drug Metab Rev,* Vol.43, No.1, pp. 27-40, ISSN 1097-9883

Kenny, J.R., Maggs, J.L., Tettey, J.N., Harrell, A.W., Parker, S.G., Clarke, S.E., & Park, B.K. (2005). Formation and protein binding of the acyl glucuronide of a leukotriene B4 antagonist (SB-209247): relation to species differences in hepatotoxicity. *Drug Metab Dispos,* Vol.33, No.2, pp. 271-281, ISSN 0090-9556

Kusuhara, H., & Sugiyama, Y. (2010). Pharmacokinetic modeling of the hepatobiliary transport mediated by cooperation of uptake and efflux transporters. *Drug Metab Rev,* Vol.42, No.3, pp. 539-550, ISSN 1097-9883

MacGregor, J.T., Collins, J.M., Sugiyama, Y., Tyson, C.A., Dean, J., Smith, L., Andersen, M., Curren, R.D., Houston, J.B., Kadlubar, F.F., Kedderis, G.L., Krishnan, K., Li, A.P., Parchment, R.E., Thummel, K., Tomaszewski, J.E., Ulrich, R., Vickers, A.E., & Wrighton, S.A. (2001). In vitro human tissue models in risk assessment: report of a consensus-building workshop. *Toxicol Sci,* Vol.59, No.1, pp. 17-36, ISSN 1096-6080

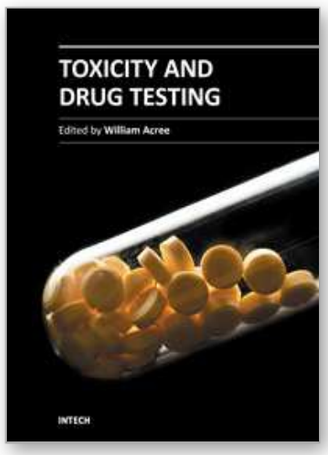
MacIntyre, A.C., & Cutler, D.J. (1988). The potential role of lysosomes in tissue distribution of weak bases. *Biopharm Drug Dispos,* Vol.9, No.6, pp. 513-526, ISSN 0142-2782

Okumura, H., Katoh, M., Sawada, T., Nakajima, M., Soeno, Y., Yabuuchi, H., Ikeda, T., Tateno, C., Yoshizato, K., & Yokoi, T. (2007). Humanization of excretory pathway in chimeric mice with humanized liver. *Toxicol Sci,* Vol.97, No.2, pp. 533-538, ISSN 1096-6080

Smith, D.A., & Obach, R.S. (2005). Seeing through the mist: abundance versus percentage. Commentary on metabolites in safety testing. *Drug Metab Dispos,* Vol.33, No.10, pp. 1409-1417, ISSN 0090-9556

Thompson, C., Sonawane, B., Nong, A., & Krishnan, K. (2007). Considerations for Applying Physiologically Based Pharmacokinetic Models in Risk Assessment, In: *Toxicokinetics and Risk Assessment*, Lipscomb, J.C., & Ohanian, E.V. (Ed.), pp. 123-140, Informa Healthcare USA Inc., ISBN 978-0-8493-3722-2, New York, NY

Ward, P. (2008). Importance of drug transporters in pharmacokinetics and drug safety. *Toxicol Mech Methods,* Vol.18, No.1, pp. 1-10, ISSN 1537-6524



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Modern drug design and testing involves experimental in vivo and in vitro measurement of the drug candidate's ADMET (adsorption, distribution, metabolism, elimination and toxicity) properties in the early stages of drug discovery. Only a small percentage of the proposed drug candidates receive government approval and reach the market place. Unfavorable pharmacokinetic properties, poor bioavailability and efficacy, low solubility, adverse side effects and toxicity concerns account for many of the drug failures encountered in the pharmaceutical industry. Authors from several countries have contributed chapters detailing regulatory policies, pharmaceutical concerns and clinical practices in their respective countries with the expectation that the open exchange of scientific results and ideas presented in this book will lead to improved pharmaceutical products.

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