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TOXICOKINETICS: AN IMPORTANT TOOL IN NEW DRUG DEVELOPMENT

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Review Article

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ABSTRACT

Toxicokinetics (TK) is generation of kinetic data for systemic exposure and toxicity assessment of the drug. These studies help us to estimate the observed toxicity to that dose. TK evaluation is very important in drug development phase in both regulatory and scientific perspective. There are several guidelines to conduct TK study in animals recommended by regulatory bodies (OECD). TK evaluation is useful in selection of dose, dosing form, alternative dosing route, evaluation of toxicological mechanism, and also used for the setting safe dose level in clinical phases. This TK studies also used to reduces the animal number (replacement, reduction and refinement). On the other hand, TK data are practically used for the purpose of drug discovery such as lead-optimization and candidate-selection. This review discussed about the principles involved in TK studies, application and importance in drug development stages and approaches to reduction the number of animals in the study.

KEYWORDS: Toxicokinetics (TK), drug development lead-optimization, OECD

Introduction

Toxicokinetics (TK) is defined by The International Conference on Harmonization (ICH) as 'the generation of pharmacokinetic data, either as an integral component in the conduct of non-clinical toxicity studies or in specially designed supportive studies, to assess systemic exposure[1]. While developing a molecule as a therapeutic agent researchers consider not only benefit but also risk associated with it. Simply it means if the safety/risk ratio is balanced or safety is more then it will be used as good therapeutic agent. Hence toxicological evaluation got more importance in drug development stages especially in preclinical stage. The need for toxicokinetic data and the extent of exposure assessment in individual toxicity studies should be based on a flexible step-by-step approach and a case-by-case decision making process to provide sufficient information for a risk and safety assessment[2]. Several guidelines have been recommended for the toxicokinetic measurements. These measurement procedures may provide a means of obtaining multiple doses pharmacokinetic data in the test species, avoidance of duplication of studies of such studies when appropriate parameters were monitored; optimum design in gathering the data will reduce the number of animals required (replacement, reduction and refinement{3R}). However this toxicokinetic data focus on the kinetics of a new therapeutic agent under the conditions of the toxicity studies themselves. Dynamic development process of a pharmaceutical product is involves continuous feed-back between non-clinical and clinical studies, no detailed recommendations required for the application of toxicokinetic data to be collected in all studies and scientific judgment should dictate when such data may be useful.

The primary objective of the toxicokinetic studies is to describe the systemic exposure achieved in animals and its relationship to dose level and the time course of the toxicity study [1]. Second, exposure data in animals should be evaluated before human clinical trials [3]. Third, choice of species and treatment regimen used in non clinical studies. Lastly, information on systemic exposure of animals during repeated-dose toxicity studies is essential for the interpretation of study results, to the design of subsequent studies and to the

human safety assessment[4]. In addition to all these sex and inter-animal variability also need to be compared, because there are some variations depending up on the species and gender. For example drugs, such as pentobarbital, morphine and methadone, female rats have a much lower liver metabolism than males, resulting in higher plasma levels[5]. A recent survey by the Japanese Pharmaceutical Manufacturers Association compared the results from 102 repeat-dose toxicity studies (ranging from one to 12 months) in mouse, rat, dog and monkey [6]. Sex differences were observed in 41 out of 92 of the studies, primarily consisting of higher exposure in female rats. Second example, species difference, clearance of nicardipine in the plasma of rats is high compared with other species, including humans[7]. Due to hepatobiliary saturation (major metabolic pathway) in dog, proxicromil (anti allergic compound) causes liver toxicity (with elevated plasma levels) but not in the rat or monkey [8].

Principles involved in toxicokinetics:

1. Quantification and extent of exposure :

The exposure might be represented by plasma (serum or blood) concentrations or the AUCs of parent compound and/or metabolite(s) and sometimes by tissue concentrations. Quantification of exposure provides an assessment of the burden on the test species and helps in the interpretation of similarities and differences in toxicity across species, dose groups and sexes. When designing the toxicity studies, the exposure and dose-dependence in humans at therapeutic dose levels (either expected or established), should be considered in order to achieve relevant exposure at various dose levels in the animal toxicity studies. Species differences in the pharmacodynamics of the substance (either qualitative or quantitative) should also be taken into consideration because sometimes it may have other effects. This information may allow better interspecies comparisons than simple dose/body weight (or surface area) comparisons.

2. Extent of exposure

Systemic exposure should be estimated in an appropriate number of animals and dose groups to provide a basis for risk assessment. Concomitant toxicokinetics may be performed either in all or a representative proportion of the animals used in the main study or in special satellite groups. Both male and female animals are utilized in the main study it is normal to estimate exposure in animals of both sexes unless some justification can be made for not so doing. Toxicokinetic data is not mandatory for studies of different duration if the dosing regimen is essentially unchanged.

3. Sampling points

In concomitant toxicokinetic studies the time points for collecting body fluids should be as frequent as is necessary, but not as frequent as to interfere with the normal conduct of the study or to cause undue physiological stress to the animals. There are also strict restrictions on blood volume available (no more than 10% of circulating volume can be taken). Sample size is typically 0.25–0.50 ml day^{-1} in rodents and up to1ml day^{-1} in non-rodents In each study, justification of number of time points should be made on the basis that they are adequate to estimate exposure. The justification should be based on kinetic data gathered from earlier toxicity studies, from pilot or dose range finding studies, from separate studies in the same animal model or in other models allowing reliable extrapolation. Sampling times vary based on the presence (or lack) of pharmacokinetic data, but are often taken 0.5, 1.0, 2.0, 4.0, 8.0, 12.0 and 24.0 h post-dose, with only the parent drug generally being measured.

4. Dose level setting

Dose level for toxicity studies is largely regulated by the toxicology findings and the pharmacodynamic responses of the test species.

At low dose levels, preferably a no-toxic-effect dose level, the exposure in the animals of any toxicity study should ideally equal or just exceed the maximum expected (or known to be attained) in patients. This ideal is not always achievable and that low doses will often need to be determined by considerations of toxicology; nevertheless, systemic exposure should be determined.

Intermediate dose levels should normally represent an appropriate multiple (or fraction) of the exposure at lower (or higher) dose levels dependent upon the objectives of the toxicity study. The high dose levels in toxicity studies will normally be determined by toxicological considerations. However, the exposure achieved at the dose levels used should be assessed.

This toxicokinetic data indicate that absorption of a compound limits exposure to parent compound and/or metabolite(s), the lowest dose level of the substance producing the maximum exposure should be accepted as the top dose level to be used (when no other dose-limiting constraint applies).

In non-linear kinetic cases a very careful attention should be paid to the interpretation of toxicological findings in toxicity studies (of all kinds). However, non-linear kinetics should not necessarily result in dose limitations in toxicity studies or invalidate the findings; toxicokinetics can be very helpful in assessing the relationship between dose and exposure in this situation.

5. Ratifying factors on study to be considered

Earlier we discussed the species and sex differences and their effect on toxicokinetics. There are other factors to be considered in this study is protein binding, tissue uptake, receptor properties and metabolic profile. Systemic exposure may be decreased by protein binding and tissue uptake. In addition, due to the metabolism there will be formation of pharmacological active metabolites, the toxic metabolites and antigenic biotechnology products metabolites.

6. Route of administration

Pharmacokinetics of a substance is greatly affected by the route of administration. For instance orally administered drugs bioavailability time is more than other routes. If the drug is intended to administer through oral route then oral toxicity should be checked. If any drug administering route is already established and new clinical route of administration is going to establish then it will be necessary to ascertain whether changing the clinical route will significantly reduce the safety margin. In this case focusing on local toxicity is essential. In this comparison of the systemic exposure to the compound and/or its relevant metabolite(s) (AUC and C_{max}) in humans generated by the existing and proposed routes of administration is required.

7. Metabolite determination

Many of the cases systemic exposure and toxic effect consider on the basis of parent drug concentration. However, there may be circumstances when measurement of metabolite concentrations in plasma or other body fluids is especially important in the conduct of toxicokinetics. They are

- If it is a 'pro-drug' and the delivered metabolite is acknowledged to be the primary active entity.
- If the compound is metabolised to one or more pharmacologically or toxicologically active metabolites which could make a significant contribution to tissue/organ responses.
- For the drugs which are extensively metabolized and the metabolite is only the quantifiable factor.

8. Statistical evaluation of data

The data should be evaluated statistically which allows assessment of the exposure. Toxicokinetic values are normally calculated as mean SD; statistical evaluation is not usually performed however, because large intra- and inter-individual variation of kinetic parameters may occur and small numbers of animals are involved in generating toxicokinetic data, a high level of precision in terms of statistics is not normally needed. Consideration should be given to the calculation of mean or median values and estimates of variability, but in some cases the data of individual animals may be more important than a refined statistical analysis of group data. If data transformation (e.g. logarithmic) is performed, a rationale should be provided.

9. Analytical methods

Regulatory authorities expects that analytical methods used to determine plasma concentrations of pharmaceuticals are of adequate sensitivity and precision[1,9]. For evaluation validated analytical methods used and conforms to Good Laboratory Practice (GLP). Analytical methods used in such studies include gas chromatography (although this is rarely used), HPLC (UV or fluorescence), LC, LC–MS, LC-MS-MS, and capillary electrophoresis (again, rarely used, and more for proteins).In addition, the number of sample time-points must be to be frequent enough to estimate exposure[1]. Generally, toxicity studies use a range of time-points and replicates to provide toxicokinetic data as we discussed earlier, although staggered and sparse sampling (to reduce animal numbers) has been reported to give accurate results[10–13]. Results are then analysed using a set curve-prediction package (e.g.WinNonLin from Pharsight; http://www.pharsight.com). For replicate designs, toxicokinetic measurements are taken at similar pre-set timepoints and the mean of the measured values is then taken to provide an estimate of drug exposure. Although staggered designs are less sensitive, they are still used by various pharmaceutical companies for rodent and/or primate studies.

Toxicokinetic studies in Preclinical stage:

Safety assessment

Generally safety of a molecule can be performed in in-vivo systems. This step is not included in the guidelines but it is very useful for the researchers to assess the systemic exposure of the molecule and its effect on it. This safety study is integral part in the central nervous system (CNS), cardio vascular system (CVS) and respiratory assessments.

Single dose and rising dose studies

These studies are often performed in a very early phase of drug development before a bioanalytical method has been developed. These studies are usually performed in rodents. Plasma samples may be taken in such studies and stored for later analysis, if necessary; appropriate stability data for the analyte in the matrix sampled would then be required. To answer specific questions raised from the initial single dose study alternatively, additional toxicokinetic studies may be required. Results from single-dose kinetic studies may help in the choice of formulation and in the prediction of rate and duration of exposure during a dosing interval. This may assist in the selection of appropriate dose levels for use in later studies. However, toxicokinetics can be assessed for some drug classes, or in screening studies (e.g.in a series of candidates or when choosing a suitable formulation). Rising-dose studies are performed in non-rodent models. Here, toxicokinetic evaluation takes place at various time-points for each new dose level. Such an evaluation is especially useful if higher-dose emesis occurs as it can reveal whether exposure to the drug still occurred.

Repeated-dose toxicity studies

To give support for phase 1 studies this study is carried out for four weeks in both rodents as well as non-rodents. The treatment regimen (Note 11) and species should be selected whenever possible with regard to pharmacodynamic and pharmacokinetic principles. This may not be achievable for the very first studies, at a time when neither animal nor human pharmacokinetic data are normally available. As we discussed earlier no rigid detailed procedures for the application of toxicokinetics are recommended in regulatory guidance documentation[1]. Toxicokinetics should be incorporated appropriately into the design of the studies. It may consist of exposure profiling or monitoring (Note 1) at appropriate dose levels at the start and towards the end of the treatment period of the first repeat dose study. The procedure adopted for later studies will depend on the results from the first study and on any changes in the proposed treatment regimen. Monitoring or profiling may be extended, reduced or modified for specific compounds where problems have arisen in the interpretation of earlier toxicity studies. These results give information on exposure, dose proportionality, sex- and species-difference, and potential accumulation and inhibition, and help to support dose-selection for subsequent studies

Performing further repeated dose studies in both rodent and non rodents up to 6-12 months enable estimation of drug and its metabolite(s) kinetic

parameter assessment as well as long term clinical exposure assessment. Another point to be considered is a few drugs shows tolerance when it is administered repeatedly.

Genotoxicity studies

Two in vitro studies and one in vivo study is essential to support development of drug [14]. In vivo investigations usually use a rodent micronucleus (bone marrow or peripheral erythrocytes) test or chromosome aberration (bone marrow cells) test. These are the well established studies for the genotoxicity evaluation. There is a regulatory expectation to demonstrate exposure to the drug either with toxicity or toxicokinetic data[1,15]. In rodents, specific toxicokinetic evaluation might not be necessary as it is possible to cross reference with toxicity studies.

Reproduction toxicity studies

Reproduction toxicity measurements are taken in studies of fertility (rat), embryo-foetal development (rat and rabbit) and peri- or post-natal development (rat).

Studies of fertility

Assessment of fertility toxicity has very important, because most of the drugs used in fertility conditions so has to strengthen at that time. Usually this can be done in rats.

In pregnant and lactating animals:

There is a regulatory expectation for toxicokinetic data in pregnant animals, although no specific guidance is given[1,16]. Data from non-pregnant animals is useful to set dose levels, and the limitation of exposure is usually governed by maternal toxicity. Toxicokinetics may involve exposure assessment of dams, embryos, foetuses or newborn at specified days. Secretion in milk may be assessed to define its role in the exposure of newborns. In some situations, additional studies may be necessary or appropriate in order to study embryo/foetal transfer and secretion in milk.

The point at which toxicokinetic evaluation is performed varies among pharmaceutical companies but often takes place in embryo-foetal studies at the beginning and end of gestation in the main study animals themselves. However, it can also occur in preliminary studies or in main studies with satellite animals.

Carcinogenicity studies:

Sometimes drugs are used for longtime for curing purposes, this may lead to the toxicity or carcinogenicity. So lifetime studies in the rodent are needed to support the long-term clinical use of pharmaceuticals[17] and non-rodents can also be used. Dose selection is usually determined as the maximum tolerated dose (MTD), which is a 25-fold AUC ratio (rodent to human), or by dose-limiting pharmacodynamic effects, saturation of absorption, or a maximum feasible dose[9]. Selection based on AUC is less common as a 25-fold ratio is often not feasible. Indeed, at the highest dose level, most drugs do not yield AUC values of more than 5–10-fold the human AUC[18]. There is a regulatory expectation for information on systemic exposure to the parent drug and metabolites[1,19]. It is recommended that monitoring should occur on a few occasions during the study, although it is not essential for monitoring to occur beyond six months[1]. However, pharmaceutical companies use various strategies for such monitoring times (e.g. Weeks 1, 13, 26 and 52, Weeks 1 and 26, or Weeks 26 and 52). It should be noted that, owing to high variability in plasma concentration, toxicokinetic data from aged rats (above one year old) are not useful for estimating exposure. Sampling times depend on available kinetic data but can range from full profile (up to 24 h) to limited time-points which are earlier stated.

Toxicokinetic studies in clinical phases:

Regulatory bodies around the world outlining that toxicity studies are necessary to support human Phase I, II and III studies, and product license application is available[3]. The magnitude of the preclinical toxicokinetic evaluation for each clinical phase varies significantly among pharmaceutical companies. For Phase I investigations the company might only generate toxicokinetic data from the four-week repeat-dose toxicitystudies. Full pharmacokinetic profile (including in vitro

metabolism studies), and toxicokinetic measurements from four- and 13-week repeat-dose toxicitystudies prior to Phase I is necessary. Toxicity assessments enable the No Observed Effect Level (NOEL) or No Observed Adverse Effect Level (NOAEL) to be established for a potential new drug, based on clinical observations, bodyweight, food consumption, clinical pathology, organ weights, necropsy examination, and histopathology. Toxicokinetic data from either NOEL or NOAEL [and subsequent toxic level(s)] can be used to give guidance to the clinical investigator by providing suitable safe starting and upper doses in the initial single-dose Phase I study. For further clinical studies using multiple dosing, toxicokinetic data from toxicity studies provide information on possible increases or decreases of drug in plasma. Cases where human plasma levels in a Phase I study are higher than in the animal study NOEL or NOAEL values need to consider the effects of different metabolism and plasma protein binding. This might result in the use of a different species in the toxicity study and/or a change of formulation to enable reassessment of safety margins.

Approaches to decrease the animal usage in toxicokinetics:

To increase the generation of toxicokinetic data it has to increase the usage of number of rodents through the use of satellite groups. As discussed earlier using of animal's number and sex is restricted as per OECD-417 guidelines even in blood samplings. For this purpose many alternative approaches has generated.

Dried blood spot technology:

Recently toxicokinetic studies in dogs by applying dried blood spot technology published and also it is in improvement stages to use this model in rodents (bar). In Pharmaceutical industry this type of methodology for toxicokinetics has been developed and combined with high performance liquid chromatography–mass spectrometry (HPLC–MS/ MS) to collect and analyse very small amounts of biofluids.

By this approach, smaller volumes of blood (typically 10–20 lL per sample) used to generate high quality toxicokinetic information than are conventionally required (200 lL for mice and 250 lL for rats). This could enable a significant reduction in rodent numbers for generation of kinetic data as serial samples can be obtained from the same animal. Toxicokinetic samples can be collected from rats already in the toxicological study, while the number of mice in a satellite group can be reduced.

Alternative approaches to animal models

Using alternative models to animal models can reduce the number of animals in confirmative studies [19-21]. Although this is not basis for the use of the chemical entity in clinical phases, but prior to human usage it should check with the animal studies.

For example carcinogenecity studies of chemical entity, alternative model like cell transformation assay used. In cell transformation Syrian hamster embryo (SHE) cells,.Balb/c 3T3 mice cells and C3H/10T1/2 (puripotent stem cells) cells can be used.In these assays, carcinogenicity of test substances is determined by measuring phenotypic changes such as cell morphology, colony growth patterns and cell adhesion induced by chemicals in mammalian cell cultures. The most widely used of these assays are the Syrian hamster embryo (SHE) assay [22], the low-pH SHE assay, the Balb/c 3T3 assay[23], and the C3H/10T1/2 assay[24]. The SHE assay is believed to detect early steps of carcinogenesis, and the Balb/c and C3H10 assays detect later carcinogenic changes.

Physiologically based pharmacokinetic (PBPK) **modeling:**

PBPK models can scientifically support risk assessment by facilitating extrapolation between species and exposure routes, and from high to low doses. Loizou et al., 2008 used this approach to understand the relationship between external and internal exposures, helping in dose response characterisation and the interpretation of biomarker levels determined in bio-monitoring studies [25].

The replacement, reduction and refinement (3Rs) of animal studies can be potentially done by using

PBPK. For example, the use of PBPK models for extrapolation of results of animal studies across species or exposure can be done by PBPK models which will results in the reduction of animal use. PBPK models can also aid in selecting the optimum dose and design of toxicokinetic sampling schemes for in vivo studies [26].

For pharmaceutical drug development, a number of generic PBPK models have been developed to predict *invivo* kinetics using *invitro* and *insilico* data, including quantitative structure–activity relationship (QSAR) techniques, on factors such as metabolism, plasma protein binding and lipophilicity [27]. This approach is referred to as invitro to invivo extrapolation, and several commercially available software packages facilitate its use, such as Gastro Plus and Simcyp [28,29]. Generic models have also been used to assess non-pharmaceutical compounds.

PBPK models accuracy promoted and improved by various factors such as development of better statistical models and methods for characterizing variability and uncertainty and improvement of databases on physiological parameters and their intra and inter-individual variation and principle guidelines for good modeling. These will offer PBPK models as powerful and cost effective tools for predicting kinetic behavior while also reduce the animal usage.

Conclusion:

Thorough toxicokinetic evaluation is important in drug development stages. This evaluation should constitute effective analytical methods having good accuracy and precision, adequate sampling, drug and metabolite(s) evaluation both in animals and humans (if necessary) and sufficient results evaluation. Toxicokinetic data is important to know the toxic response(s) to that of drug exposure obtained in drug development stages (preclinical) and it is used to set safe dose for clinical use of new drugs and also it is useful in the understanding of differences in responses or sensitivity between individual animals, genders, species or life stages, and supporting the extrapolation of findings in experimental animals to humans. Kinetic data can also support mode-of-action analysis and extrapolation across exposure routes. Now a day toxicokinetics used in other areas also with other areas of pharmacokinetics. Such as toxicokinetic assessments using biomarkers, are used earlier in screening studies, provide data for allometric species scaling, and even play a role in measuring drug levels in non-plasma samples (tissues, urine and bile). Even though toxicokinetic evaluation is only a small part of the process of understanding the fate of a drug, it has a vital part in drug development – a role that proceeds to advance.

References:

1. CPMP/ICH/384/95: Operational June 1995. Toxicokinetics: guidance for assessing systemic exposure in toxicology studies. Also known asICH Topic S3A (see http :// www. emea.eu.int)

2. Kate, R., Case, D.E., Hakes, H., Nod, K., Salami, F., Horii,I., Mayahara, H.,Cayeb n, M.N., Marriott, T.B., Igarashi, T., Toxicokinetics: its significance and practical problems.J.Toxicol.Sci.18:211-238;(1993).

3. CPMP/ICH/286/95, modification: operational November 2000. Nonclinical safety studies for the conduct of human clinical trials for pharmaceuticals. Also known as ICH Topic M3 (seehttp://www.emea.eu.int)

4. CPMP/SWP/1042 Corr: operational October 2000. Note for guidance on repeated dose toxicity (see http://www.emea.eu.int)

5. LaDu, B.N. Fundamentals of Drug Metabolism and Drug Disposition, Robert E Krieger Publishing Company, New York, pp.528 (1979)

6. Igarashi, T. and Sekido, T. Case studies for statistical analysis of toxicokinetic data. Regul. Toxicol. Pharmacol. 23:193–208; (1996).

7. Sjoberg, P. Toxicokinetics in preclinical safety assessment views from the Swedish Medical Products Agency. Drug Inf. J. 28:151–157;(1994).

8. Smith, D.A.Pharmacokinetics and pharmacodynamics in toxicology. Xenobiotica 27: 513–525;(1997).

9. CPMP/ICH/383/95: Operational March 1995. Dose selection for carcinogenicity studies of

pharmaceuticals. Also known as ICH Topic S1C (see http://www. emea .eu int)

10. Dahlem, A.M. et al. Concomitant toxicokinetics: techniques for and interpretation of exposure data obtained during the conduct of toxicology studies. Toxicol. Pathol. 23:170–178; (1995)

11. Pai, S.M. Fettner SH, Hajian G, Cayen MN and Batra Characterization of AUCs from sparsely sampled populations in toxicology studies. Pharm. Res. 13:1283–1290; (1996)

12. Tse, F.L. and Nedelman, J.R. Serial versus sparse sampling in toxicokinetic studies. Pharm. Res. 13:1105–1108;(1996)

13. Van Bree, J. Nedelman J, Steimer J.L, Francis T, RobinsonW, Niederberger W Application of sparse sampling approaches in rodent toxicokinetics: prospective view. Drug Inf. J. 28: 263–279; (1994).

14. CPMP/ICH/174/95: Operational March 1998. Genotoxicity: a standard battery for genotoxicity testing of pharmaceuticals. Also known as ICH Topic S2B. (see http://www.emea.eu.int)

15. CPMP/ICH/141/95: Operational April 1996. Genotoxicity: guidance on specific aspects of regulatory genotoxicity tests for pharmaceuticals.Also known as ICH Topic S2A. (see http://www.emea.eu.int)

16. CPMP/ICH/386/95: Operational March 1994. Reproductive toxicology: detection of toxicity to reproduction for medicinal products. Also known as ICH Topic S5A (see http://www.emea.eu.int)

17. CPMP/ICH/140/95: Operational July 1996. Guideline on the need forcarcinogenicity studies of pharmaceuticals. Also known as ICH Topic S1A (see http://www.emea.eu.int)

18. Morgan, D.G. Kelvin AS, Kinter LB, Kerns CJ and Rhodes G.The application of toxicokinetic data to dosage selection in toxicology studies. Toxicol. Pathol. 22:112–123; (1994)

19. OECD, Test guideline 417: toxicokinetics. OECD guidelines for the testing of chemicals.(1984).

20. OECD Test guideline 426: developmental neurotoxicity study. OECD guidelines for the testing of chemicals (2007)

21. OECD draft guidance document on the design and conduct of chronic toxicity and carcinogenicity studies, supporting TG 451, 452 and 453. Draft ENV/JM/TG(2009)

22. Mauthe R.J, Bunch R. Gibson D.P and Custer. L. The Syrian Hamster Embryo (SHE) Cell Transformation Assay: Review of the Methods and Results. Toxicol Pathol 29: 138-142; (2001).

23. Ayako Sakai BALB/c 3T3 cell transformation assays for the assessment of chemical carcinogenicity AATEX 14:367-373 (2007)

24. Dunkel VC, Schechtman LM, Tu AS, Sivak A, Lubet RA and Cameron TP Interlaboratory evaluation of the C3H/10T1/2 cell transformation assay. Environ Mol Mutagen 12(1):21-31; (1998)

25. Loizou, G. et al. Development of good modelling practice for physiologically based pharmacokinetic models for use in risk assessment: the first steps. Regul.Toxicol. Pharmacol. 50:400–411;(2008)

26. Bouvier d'Yvoire, M. et al., 2007. Physiologically-based Kinetic Modelling (PBPK Modelling): meeting the 3Rs agenda. The report and recommendations of ECVAM Workshop 63. Altern. Lab. Anim. 35, 661–671;(2007)

27. Brightman, F.A. et al., 2006a. Application of a generic physiologically based pharmacokinetic model to the estimation of xenobiotic levels in human plasma. Drug Metab. Dispos. 34, 94–101.

28. Parrott, N., Lave, T., 2008. Applications of physiologically based absorption models in drug discovery and development. Mol. Pharm. 5, 760–775.

29. Shiran, M.R. et al., 2006. Prediction of metabolic drug clearance in humans: in vitro in vivo extrapolation vs allometric scaling. Xenobiotica 36, 567–580.

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