Regulatory Toxicology and Pharmacology 67 (2013) 325–334

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Proper knowledge on toxicokinetics improves human hazard testing and subsequent health risk characterisation. A case study approach

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ARTICLE INFO

Article history:
Received 17 April 2013
Available online 17 September 2013

Keywords:
ADME
Toxicokinetics
Absorption
Bioavailability
AUC
Route-to-route extrapolation
Species selection
Risk characterisation
Risk assessment

ABSTRACT

In the current EU legislative frameworks on chemicals safety, the requirements with respect to information on general kinetic parameters (absorption, distribution, metabolism and excretion or ADME) or integrated toxicokinetic parameters (TK, i.e. plasma concentration-time curve, area under the curve etcetera) in humans and experimental animals vary widely. For agrochemicals and cosmetics, there are regulatory requirements whereas for other frameworks, such as food ingredients, biocides, consumer products and high production volume chemicals (REACH) there are very little or no requirements.

This paper presents case studies that illustrate the importance of ADME and TK data in regulatory risk characterisations. The examples were collected by interviewing regulatory risk assessors from various chemicals (non-pharmaceutical) frameworks.

The case studies illustrate how (1) applying ADME/TK in an early phase of toxicity testing can be used to improve study design and support the 3R-goals and how (2) increased use of ADME/TK data can improve the final risk assessment.

1. Introduction

1.1. Importance

For human pharmaceuticals, information on toxicokinetics (TK) has been an important part of the safety assessment for decades (ICH, 1995; Ploemen et al., 2007). Toxicologic pathologists deal with toxicokinetics instead of pharmacokinetics. Toxicokinetics is defined as the description of the concentration of a compound in plasma (or serum or whole blood) with respect to time, based on a limited number of plasma samples, as a measure for internal exposure within a toxicity study (Ploemen et al., 2007). It is the general framework in which during toxicity testing kinetics is studied in order to assess systemic exposure within toxicity studies.

Taking species, sex and life-stage related differences in toxicokinetics into consideration is pivotal, from research and development (R&D) to preclinical safety testing to phase I and II in the clinical trials. Toxicokinetics is an important requirement as drugs are deliberately taken (patients) with or without prescription by a physician. Usual dosages are significantly higher than daily chemicals exposure in other scenarios (worker, consumer, general population).

1.2. Differences between regulatory frameworks

Data requirements under the majority of non-pharmaceutical regulatory frameworks (chemicals frameworks such as consumer products, food additives, biocides and industrial chemicals) do not consistently include toxicokinetics in general or one of its underlying processes absorption, distribution, metabolism and excretion (ADME). Toxicokinetics is defined as a description of the time-dependent fate of a substance within the body, i.e. by definition toxicokinetics is the integration of the individual processes A, D, M as well as E.

From a scientific point of view, systemic exposure (e.g. AUC or C,t-curve as such) as measurable outcome of the ADME processes is a determining factor for the possible systemic adverse health effects. Without internal exposure, systemic toxicity is quite unlikely. As such, information on systemic exposure might have large value in designing toxicity studies (Saghir et al., 2012; Creton et al., 2012) as well as in risk characterisation. Concrete data are information on the rate, extent and duration of systemic exposure across doses, species (including human), strains, sex and life-stages (Creton et al., 2012).

Abbreviations: ADME, absorption, distribution, metabolism and excretion; AUC, area under the blood/plasma concentration time curve; BMDL, benchmark dose lower limit; NOAEL, no-observed-adverse-effect level; TK, toxicokinetics; C,t-curve, blood/plasma concentration-time curve.

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http://dx.doi.org/10.1016/j.yrtph.2013.08.010

1.3. What is available?

From the perspective of protocols on study design for ADME studies, some important work has been done. In its Environment, Health and Safety (EHS) Programme, OECD has issued three Test Guidelines: Test Guideline (TG) 417 'Toxicokinetics' as revised in 2010, TG 427 'Dermal absorption – *in vivo* method' and TG 428 'Dermal absorption – *in vitro* method' (OECD, 2010, 2004a,b, respectively). One or more of these OECD TG studies and endpoints are required in some EU regulatory frameworks such as the agrochemicals and the cosmetic ingredients. Moreover, a considerable amount of attention is paid towards the inclusion of information on (dermal) absorption and oral and dermal bioavailability such as in the Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation of the EU Scientific Committee on Consumer Safety (SCCS, 2012a).

Absorption and bioavailability: It is noted that absorption and bioavailability are not always used properly (e.g. when an absorption figure is used as if it were bioavailability without noting possible pre-systemic/firs-pass metabolism). This sometimes leads to confusion. Absorption can reflect to the process as such (i.e. the crossing of an outer layer) as well as to an endpoint (e.g. absorption is 35%). Bioavailability is a somewhat more complex endpoint that covers absorption and (first-pass) metabolism and is usually expressed as a number between 0 and 1 (not and completely bioavailable at the site of measurement, respectively).

SCCS tends to use the term '(animal) oral bioavailability' (leaving unspecified whether metabolism is explicitly included) and '(human) dermal absorption'.

In case it was not clear or could not be made clear without extensive explanation, which of the two to use, 'absorption/bioavailability' was used.

1.4. Practice

In many cases, these ADME and TK endpoints are not generated before the final phase of the chemical safety assessment. As such and in practice, the potential or power of these kinetic endpoints to be used in early and iterative hazard testing strategies (integrated testing strategies) is quite low (Barton et al., 2006). Information on ADME/TK acquired in an early phase of chemical safety assessment can for example be used to select a proper dosing regimen for toxicity testing (in case of non-linear kinetics occurring at high doses) or to select the most appropriate model species (in case one species turns out to be a clear outlier with respect to kinetics). In contrast to pharmacokinetics studies where ADME processes are assumed to be first-order and linear, toxicokinetics must also consider zero-order and nonlinear processes in interpretation (Dixit and Ward, 2007).

Furthermore, generating ADME/TK data during the final phase of the safety assessment may also hamper a proper regulatory risk characterisation as the ADME/TK data delivered to the risk assessor may be insufficient and/or irrelevant. It is probably much more relevant to generate ADME/TK data in parallel with the toxicity studies. The same holds for basic parameters derived from sampling the same animals that are in the toxicity study. ADME/TK data that were collected completely separately may have been examined under different conditions with respect to dose, concentration, route, strain, life-stage etc. than relevant for the critical health effect study and/or the human exposure situation. As a consequence, ADME/TK data may have to be acquired again or a health effect study has to be repeated following another design (e.g. other species, other route). The latter possibility is scrutinised in this paper. In other words, are there concrete examples that illustrate that the timing of generating ADME/TK data is essential for proper regulatory assessment? And how can we learn from these findings in order to improve risk characterisation as well as the preceding hazard testing strategy and change the latter to an iterative, integrated testing strategy?

1.5. Progress in health effect test guidelines

The recently adopted OECD TG 443 (Extended One-Generation Reproductive Toxicity Study – EOGRT) is probably the first OECD TG that is specifically addressing the importance to take into account (existing) information on ADME/TK (OECD, 2011a). It states that 'The review of existing information is important for decisions on the *route* of administration, the choice of the *vehicle*, the selection of animal *species*, the selection of *dosages* and potential modifications of the dosing schedule. ...., all the relevant available information on the test substance, i.e. .... (including *species-specific metabolism*), ..., *in vitro metabolic* processes and ..... should be taken into consideration in planning the EOGRT'.

It was also the OECD that issued Guidance Document No. 97 as a detailed review on the use of metabolising systems for *in vitro* testing of endocrine disruptors (OECD, 2008). And OECD Guidance Document 117 on the current implementation of internal triggers in TG 443 (EOGRT) in the US and Canada (OECD, 2011b) notes that consideration should be given to issues such as TK and/or metabolism and human exposure information in order to decide whether second generation testing is necessary.

1.6. Industries perspective

Within the framework of agrochemical risk assessment, already in 2006 the Agricultural Chemical Safety Assessment (ACSA) Technical Committee of the ILSI Health and Environment Sciences Institute (HESI) proposed a tiered testing strategy which incorporates ADME/TK already in the early phase of toxicity assessment (Barton et al., 2006). This testing strategy is not yet completely implemented in a toxicity test guideline or a regulatory driver, such as the new EC agrochemicals regulation 2009/1107 (EC, 2009), even though this regulation includes some requirements on ADME/TK. As a follow-up to Barton et al. (2006), case studies were presented recently by Creton et al. (2012). They highlighted the importance of TK in facilitating study design and supporting chemical risk characterisation. The paper of Creton et al. (2012) focused mainly on opportunities for the use of TK to support chemical evaluation (dose selection, study interpretation and development of a Mode of Action hypothesis) from the perspective of the industry. In addition, Saghir et al. (2012) nicely illustrates an integrative procedure to implement TK in an integrated testing strategy (i.e. the use of TK information in design and dose selection) within an agrochemical company.

1.7. Better study design and the 3R goals

As such, these uses of TK can also support the 3R's (replacement, reduction and refinement of animal use). TK information could help to avoid excessively high doses which could result in unnecessary suffering in experimental animals and it can help choosing the most appropriate dosing regimen for the health effect testing (linear dose range; gavage or diet). For example, studying ADME/TK in an early phase of safety testing could provide information on potential non-linear kinetics at high dose-levels due to saturation of e.g. absorption processes. This information can be used to select the proper dosing regimen for the toxicity study (i.e. not selecting irrelevant high doses).

Table 1
Use of information on ADME/TK in risk assessment strategies.

Information on rates and extent	Regulatory toxicity testing	Regulatory risk characterisation
Pre-systemic metabolism	Species selection	Inclusion or exclusion of particular species
Absorption	Route selection	Route-to-route extrapolation Interspecies extrapolation High to low dose extrapolation
Distribution	Assessment potential delayed toxicity[a]	Incorporate in Mode of Action analysis framework
Metabolism	Species selection Sex selection Selection of life-stage	In- or exclusion of particular species Assess validity of route-to-route extrapolation
Excretion	Species selection	Assess relevance of particular species used for effects testing
C,t-curve, AUC	Species selection (wide linearity) Selection dose range (linear range) Assess systemic diurnal exposure	Saturating dose levels hamper high-to-low dose extrapolation Use AUCs (man and animal), not default assessment factors

TK-information furthermore minimises the risk of follow-up studies required to understand what is happening at irrelevant high doses. As such, this information avoids irrelevant study design and thus unnecessary animal testing. In an integrated approach, generation of TK without using additional animals can help to select the most appropriate animal species (for further testing) and to interpret results from initial toxicity testing and thus improve the quality of the risk characterisation (Saghir et al., 2012).

1.8. Setup

This paper focuses on the non-pharmaceutical regulatory frameworks and challenges the essential value that knowledge on ADME and kinetics can have for the risk assessments within these frameworks. The importance and the potential of TK knowledge to improve chemical risk assessment is assessed from a regulatory perspective. Examples of use of ADME/TK data in regulatory risk characterisations are presented and discussed for their illustrative value.

In Table 1, a limited overview is presented on the most important use categories of specific information on ADME and/or TK in toxicity testing and risk characterisation. Both papers (Creton et al., 2012; Saghir et al., 2012) were written from an industries perspective. The current paper provides a more regulatory perspective. What kind of cases are regulatory risk assessors confronted with? Can industrial and regulatory risk assessors as well as regulatory authorities learn from it? What are the messages to be learnt from case studies in the chemical safety assessment arena?

2. Approach

Within a period of about 1 year (2011–2012), risk assessors from national agencies, knowledge institutes and contract research organisations in the Netherlands were approached by the authors – being experienced in the use of ADME/TK in hazard testing strategies as well as risk characterisation questions – with the aim to increase awareness of the possibilities that ADME/TK have to improve human risk assessment. All respondents were active as member of an EU or international scientific committee, panel or working group within the framework of toxicological risk assessment. During these dialogues, information on the (potential) role of ADME/TK in risk assessment (from hazard identification to risk characterisation) was presented towards the interviewed panellists. Vice versa, information on the details of the risk assessment procedures within the specific panels and regulatory frameworks was collected from the interviewee. In addition, the authors collected examples that they came across in various settings (other projects, symposia, seminars, public literature).

During the discussions with the panellists, some more theoretical as well as practical well-known examples were presented by the authors briefly to illustrate the potential role of ADME/TK information during hazard testing and risk characterisation. This opened the floor for further discussion and caused practical examples to be presented from within the framework of the panellists. It turned out that already in relative simple situations, information on ADME/TK can be very helpful and more strongly, even necessary for an appropriate risk characterisation. Some examples required a more detailed level of understanding of ADME/TK as such or of its practical use in risk characterisation e.g. when computational kinetic modelling was involved. Five of the most straightforward examples that were brought up are presented here as case studies. As it was not the intention to critically review these cases, details may have been left out in order to streamline the presentation of the case examples as such.

3. Results five case studies

From the interviews held, up to ten examples from four different risk assessment frameworks (i.e. cosmetic ingredients, food additives, plant protection products, food contact materials) were collected. After full evaluation of these cases, two of them were rejected because they related to computational kinetic modelling and would raise a sort of questions that are regarded outside the scope of this paper (e.g. questioning of assumptions on parameter values). In addition, one example was excluded as the formal risk assessment conclusion made for this case raised questions. Discussing previous conclusions was regarded expedient. Lastly, too complex or unclear examples (two in total) were also excluded, as they would require a level of detail to present the specific case that is beyond the scope of this paper. This selection process resulted in a total of five examples, originating from the risk assessment frameworks of cosmetic ingredients, food additives and plant protection products.

3.1. Case studies 1, 2, 3: cosmetic ingredients

In this section, a few examples from the regulatory framework of the EU Cosmetics Directive will be described. In the European Union, currently health and safety assessment of specific classes of cosmetic ingredients is the responsibility of the Scientific Committee on Consumer Safety[1] (SCCS) of the Health and Consumer Protection Directorate-General (DG SANCO).

These evaluated cosmetic ingredients concern substances on Annex II (forbidden substances) and Annex III (restrictions) and chemicals intended as colouring agents (Annex IV), preservatives (Annex VI) and UV-filter (Annex VII). The SCCS takes into account

[1] Formerly known as Scientific Committee on Consumer Products (SCCP) and before that as Scientific Committee on Cosmetic Products and Non-Food Products (SCCP–NFP).

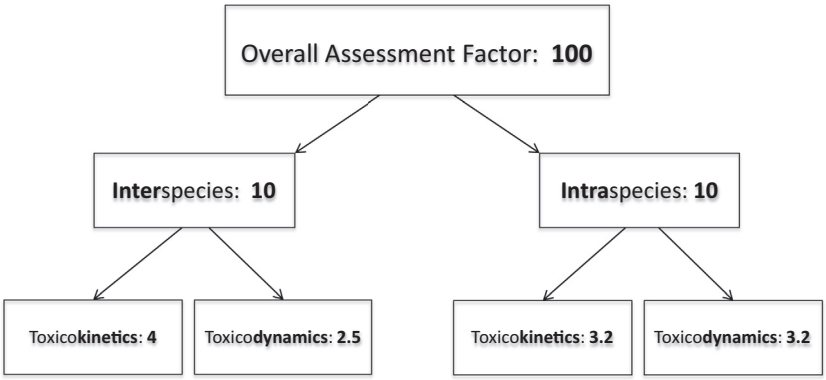

Fig. 1. Build-up of the required margin of safety from individual assessment factors that should compensate for possible interspecies differences and intraspecies variability, both with respect to toxicokinetics as well as toxicodynamics.

the intended exposure scenario and all relevant toxicity studies in a Margin of Safety approach to come up with a conclusion whether the intended uses are safe or not. This evaluation is published in an opinion which is taken forward to DG SANCO for risk management purposes.

In these evaluations according to the Notes of Guidance (SCCS, 2012a), a human internal dose called systemic exposure dose (SED) is calculated (mg/kg bw/d) and compared to the point of departure (POD) which can be a NOAEL (no-observed-adverse-effect level) or a BMDL (lower 95% confidence limit of the benchmark dose), both in mg/kg bw/d as well, for the most relevant toxicological endpoint in an animal toxicity study. This results in the Margin of Safety (MoS):

$$MoS = \frac{NOAEL}{SED} \quad \text{or} \quad MoS = \frac{BMDL}{SED}$$

In general, an overall minimal MoS of 100 is required as it should compensate for possible interspecies differences and intraspecies variability in kinetics as well as dynamics. In general risk characterisation frameworks, these differences are compensated for by various assessment factors as depicted in Fig. 1 (WHO, 2005).

The human systemic exposure dose (SED) is an internal dose per se. By default, the NOAEL and the BMDL are internal doses as well: the SCCS currently assumes 50% absorption in the underlying oral toxicity study[2]. In case of indications that absorption underlying the NOAEL or BMDL is low, the route- and species-relevant absorption data may be used to convert the NOAEL or BMDL to an internal dose in mg/kg bw/d that is more data-informed (away from the default approach).

It is noted that the AUC (Area Under the Curve of the blood/plasma concentration-time curve) at the BMDL/NOAEL in the toxicity study would be a preferable dose metric estimation of the internal dose from a scientific point of view. However, in that case the SED should also be available as an AUC at relevant human exposure scenario conditions; the MoS can then be calculated as the animal-to-human AUC ratio. Comparison of AUCs is preferred since it does not only take species- and route-specific differences in absorption into account but also species- and route-specific differences in (rates of) metabolism and excretion. Three examples are presented below showing how information on ADME/TK contributed to the risk assessment by SCCS or how lack of this information hampered a risk assessment. One example is on interspecies extrapolation (rat to human) and route-to-route extrapolation and the other two are about route-to-route extrapolation.

[2] In previous evaluations, SCCS used 100% oral absorption as default factor in case of lack of substance-specific absorption data (SCCS, 2010a).

Case study 1: p-Phenylenediamine

In its opinion on the hair dye ingredient *p*-phenylenediamine (PPD) the SCCP followed the Notes of Guidance (SCCNFP, 2003) to come up with a Margin of Safety (MoS) with respect to the use as chemical ingredient for hair-dyes (SCCP, 2006). A human internal exposure or systemic exposure dose (SED) of 0.052 mg/kg bw/d was calculated based on dermal absorption data from scalp application as measured in a human volunteer study (see Table 2). The SED was compared to a NOAEL of 4 mg/kg bw/d (rat, oral, 90-days) and resulted in a MoS of 77. This resulting MoS was smaller than the required minimal MoS of 100.

In the same opinion in 2006, a safety evaluation was performed that included more complete pictures of absorption, metabolism, distribution and excretion: plasma concentration-time curves in orally administered rats and human volunteers exposed via the skin as well as the respective, accompanying AUCs. AUC and Cl (clearance) relate directly to the steady-state body burden during chronic administration (WHO IPCS, 2005). When the effect under consideration is due to reversible interaction of the compound with a pharmacological target (e.g., a receptor or ion channel) or due to direct irritation, then the concentration of the substance rather than total intake should determine the magnitude of the effect i.e., the C_{max} is likely to be more relevant than AUC (Solecki et al., 2005). As there are no indications that there is a direct interaction of the substance with a specific target, AUC would be the preferred dose-metric for this specific case.

Internal exposure (expressed as AUC; PPD equivalents per ml blood plasma * h) in the relevant oral rat 90-days study was calculated based on a kinetics study administering a single dose at the NOAEL level of 4 mg/kg bw/d. This was compared to the AUC as measured in a human volunteer study that included application of a hair dye containing ^{14}C-labelled PPD. This resulted in a MoS of 16.3. When calculating the MoS as the rat-to-human AUC ratio the assessment factor of 4 for interspecies differences in kinetics (see Fig. 1) was considered superfluous (i.e. equal to 1) and thus the minimal MoS becomes 25. In their 2006 opinion the resulting MoS of 16.3 was therefore still regarded not sufficient.

In a recent opinion in 2012, SCCS evaluated new studies and arguments submitted by the industry (Cosmetics Europe, formerly known as COLIPA) regarding the establishment of the NOAEL as well as new data on human plasma kinetics and estimation of area that is exposed during hair dying (SCCS, 2012b). As a result, the NOAEL was increased from 4 to 8 mg/kg bw/d and the SED lowered from 0.052 to 0.04 mg/kg bw/d (Table 2). Using the conventional approach (100% absorption in the oral toxicity study) now resulted in a MoS of 200 in the conventional

Table 2
Summary of SCCS opinions on *p*-phenylenediamine.

Opinion	Relevant animal toxicity study	Human relevant exposure scenario	Safety assessment		Conclusion of SCCS
	POD (ext) AUC at POD	Human (int) = SED[a] AUC Human	Minimal MoS	Actual MoS	
2006	4 mg/kg bw/d	0.052 mg/kg bw/d	100	77	Conventional approach MoS not sufficient
	10.8 μg-eq*h/ml[b]	0.66 μg-eq*h/ml	25	16.3	Toxicokinetics-based approach MoS not sufficient
2012	8 mg/kg bw/d	0.04 mg/kg bw/d	100	200	Conventional approach Overruled by TK-based approach[d]
	33.0 μg-eq*h/ml[c]	1.415 μg-eq*h/ml	25	23	Toxicokinetics-based approach MoS borderline, but exposure intermittent: No concern

[a] Human SED is calculated by taking human penetration (per cm^2), multiplying by area exposed, dividing by human body weight (60 kg).
[b] Determined in rat study orally administered (gavage) 4 mg/kg bw ^{14}C-PPD.
[c] Determined in rat study orally administered (gavage) 6.45 mg/kg bw ^{14}C-PPD. Normalised to the dose, the AUC was slightly higher than that in rats administered 4 mg/kg bw/d, possibly indicating slower excretion at higher dose level (saturation).
[d] This MoS, based on the conventional approach (i.e. comparison animal external NOAEL with human SED), would be sufficient in general. However, as the SCCS opinion in 2006 showed that the conventional default approach overestimates the MoS, the conventional approach was followed up directly by the TK-based approach in their 2012 opinion as well.

MoS approach. This compares to a minimal MoS of 100. As it was known from the evaluation in 2006 that the default approach that neglects lower oral absorption underestimates the risk (overestimation of MoS), SCCS in its second line of evaluation, took into account measured kinetic data (the AUCs) from rat and human volunteers. This resulted in a predicted AUC at the rat oral NOAEL of 33 μg-eq*h/ml and a measured AUC in human volunteers of 1.4 μg-eq*h/ml. Using these kinetic data, a MoS of 23 was calculated. As this is close to the minimal MoS of 25 (leaving the default assessment factor for interspecies differences in kinetics out as measured kinetic data were used) it was regarded a borderline situation. Taking into account the fact the hair dying is an intermittent exposure scenario, the use of PPD in hair dyes was concluded as 'no concern'.

Lessons learned

This example shows that species- and route-specific kinetic data (in this case AUCs of the parent compound) can be crucial for a proper risk characterisation. Comparison of the default approaches (default 100% oral absorption and use of default interspecies assessment factor) and the approach that includes species- and route-specific internal exposure (TK-based approach) exhibits that the first approach results in underestimation of the risk (overestimation of the MoS).

Case study 2: camphor benzalkonium methosulphate

A few years ago, the SCCP published an opinion on the safe use of the UV-filter camphor benzalkonium methosulphate (SCCP, 2008). In this opinion, a MoS of 109 was calculated for the use of 3% camphor benzalkonium methosulphate in sun protection and other cosmetic products. This MoS was based on a NOAEL of 300 mg/kg bw/d for Mexoryl SO (a 29.7% solution of camphor benzalkonium methosulphate) for effects in the GI tract (rat, 90-day, oral). This corresponds to a NOAEL of 89.1 mg/kg bw/d for the active ingredient camphor benzalkonium methosulphate. Furthermore, data from an *in vitro* dermal absorption study using human skin samples (according OECD 428) indicating a mean dermal absorption of camphor benzalkonium methosulphate of 0.65 ± 1.04 μg/cm^2 were applied to calculate an SED of 0.819 mg/kg. Based on the available data, the committee considered a concentration of 3% as a UV filter in cosmetic products as safe.

Using common route-to-route extrapolation (De Raat et al., 1997; Rennen et al., 2004) in the MoS approach the human systemic exposure dose after dermal exposure (the SED) was compared to the rat external oral dose (the NOAEL). A direct comparison of the human SED, calculated from dermal exposure, to the rat oral NOAEL implicitly assumes complete oral bioavailability (100% absorption, 0% first-pass metabolism). In cases where there are indications that oral bioavailability is low (low absorption and/or high degree of first-pass metabolism), the NOAEL from the oral toxicity study must be corrected by the fraction of oral bioavailability in order to estimate an appropriate internal dose to be compared with the SED. This requires information on the extent of oral bioavailability (SCCS, 2010a; SCCS, 2012a). This was not yet taken into account in the opinion presented above in which 100% oral absorption in the rat study was assumed (SCCP, 2008).

Pre-systemic and first-pass metabolism: Although dependent on the context, first-pass metabolism is defined as the metabolism (biotransformation) occurring before the compound (or its first-pass metabolites) reach(es) the systemic circulation. For oral exposure this usually refers to metabolism in the GI-tract epithelium lining and to hepatic metabolism. For dermal exposure, this usually refers to skin metabolism and for airway exposure to metabolism in the airway epithelium. Pre-systemic metabolism, especially after oral exposure, has a somewhat wider coverage. Usually, it includes metabolism in the gut as well

Recently, in an addendum the SCCS concluded that risk characterisation using a MoS calculation based on route-to-route extrapolation could not be performed for camphor benzalkonium methosulphate (SCCS, 2011). Member States had submitted information which indicates low oral bioavailability (low absorption and/or high degree of first-pass metabolism) of the substance: (1) only low levels of parent compounds could be detected in the blood following oral administration in a 90-day repeated dose toxicity study, and only at the highest dose level (1000 mg/kg bw/d Mexoryl SO) and (2) in a dose-range finding study where camphor benzalkonium methosulphate was administered subcutaneously, lethality (2 of 3 dosed animals) was observed at external dose levels where no effects were seen in the oral study (300 mg/kg bw/d Mexoryl SO). Quantification of the extent of oral bioavailability was however not possible as the submitted information was regarded insufficient.

The SCCS concluded that, with the indications for limited oral bioavailability, an assessment relying on route-to-route extrapolation

from the results of oral repeated dose toxicity studies could not be performed unless quantitative information on the extent of oral bioavailability would become available, allowing transfer of the external NOAEL to a systemic NAEL (NAEL as the systemic counterpart is not 'observed' but 'calculated' based on the NOAEL).

Case study 3: bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane

In 2010 a SCCS-opinion on the safe use of bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane as a new UV-filter in sunscreen products was published (SCCS, 2010b). A NOAEL of 1000 mg/kg bw/d (doses tested 100, 300 and 1000 mg/kg bw/d), derived from a 90-day oral rat study, was available for MoS calculation. Its physicochemical properties (very low water solubility: 0.0001 mg/L; high MW: 741.12 g/mol; high log K_{ow}: 9.4) indicated low bioavailability for the substance for all relevant exposure routes (oral, dermal, inhalation). The results from satellite groups for bioavailability of the 90-day *oral* repeated dose study showed that at least a small fraction of the administered substance becomes systemically bioavailable and that accumulation does not occur. The measured plasma concentrations were very low albeit just above the lower limit of quantification which might have hampered the reliability. Also, the plasma was analysed up to 10 months after sampling. Lastly, only the parent compound was measured so no information on metabolites was available. Nevertheless, taking all information into consideration, albeit low, the extent of oral bioavailability could not be determined from this study.

In this case, while low oral systemic availability might be a favourable property of substances being intended to be used as UV filters, the SCCS concluded that the submitted data are not appropriate for the calculation of a MoS based on an oral NOAEL, as an appropriate correction factor for the limited oral bioavailability cannot be derived. An oral bioavailability of at least 13.4% would be needed to obtain a MoS of 100 to ensure safe use of bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane.

Case studies 2 and 3: lessons learned

These case studies show that without quantitative data on (oral) bioavailability (absorption and/or first-pass metabolism), an MoS-based approach is hampered. Oral bioavailability should have been investigated more thoroughly in a separate oral absorption study or in the oral *in vivo* toxicity studies themselves in parallel groups (Creton et al., 2012). As an alternative, a safety assessment avoiding the need for route-to-route extrapolation can be done if data from a valid 90-day dermal repeated dose study will be available. However, these studies are no longer permitted in the EU.

3.2. Case study 4: food additives

β-Carotene

In the EU, safe use of food additives, nutrient sources and other substances deliberately added to food (excluding flavourings and enzymes) is currently the responsibility of the European Food Safety Authority's (EFSA) Panel on Food Additives and Nutrient Sources Added to Food (ANS). Before EFSA was founded in 2002, the EC Scientific Committee on Food (SCF) was responsible. Worldwide, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has an important role in this respect. From within this regulatory framework, an interesting example of species differences in pre-systemic metabolism and the consequences for human risk assessment was presented. It concerns the chemical β-carotene and its use as food colouring agent and food supplement.

In 1975, the SCF endorsed the ADI of 0–5 mg/kg bw/d that was previously established by JECFA for the sum of various carotenoids, including β-carotene (group ADI). However when β-carotene was re-evaluated by the SCF in 2000 upon a request of the European Commission, the SCF decided to withdraw the group ADI of 0–5 mg/kg bw. There was insufficient scientific basis to set a new ADI. The group ADI of 0–5 mg/kg bw was based on rodent studies which were considered to lack relevance for human risk assessment. Rodents appear to convert β-carotene to vitamin A much better than humans in the gut. Nevertheless, there were no indications that intakes of 1–2 mg/day consumed as food additives (i.e. the estimated average exposure from β-carotene and related carotenoids, used as food additives) in the context of the overall dietary intake of β-carotene are harmful. Stating this, the SCF decided that food additive uses of β-carotene and related carotenoids permitted at that time to be temporarily acceptable (SCF, 2000).

This decision was confirmed under a standard re-evaluation program for food additives[3] (ANS, 2012). The ANS Panel confirmed the species specificity. In rodents, absorption of intact β-carotene (bioavailability) is very low if not zero. Large quantities are converted in the gut to vitamin A. In contrast, in man, 20–75% of the β-carotene ingested is absorbed intact. The ANS panel dismissed rodents as suitable models for evaluating the bioavailability and effects of β-carotene in human.

Lessons learned

One of the important lessons to be learned from this case study is that differences in kinetics between rat and human can vary to a great extent. More attention should be paid to this aspect. Crucial species-specific differences in the kinetics of a chemical may be simply caused because gut microflora is different in rodents compared to humans. In an ideal world, before starting a repeated dose toxicity study, (pre)systemic metabolism and absorption should be screened for. This approach would enable data-informed decisions with respect to the species of choice for toxicity testing and would prevent erroneous risk assessments and risk management decisions that are based on irrelevant data.

3.3. Case study 5: plant protection products

2,4-dichlorophenoxy acetic acid (2,4-D)

The EU Scientific Committee on Plants (SCP) discussed the issue of species specificity of systemic repeated dose toxicity in an evaluation of the herbicide 2,4-dichlorophenoxy acetic acid or 2,4-D (SCP, 2001). At the basis of this evaluation was the question: "Can the Committee comment on the adequate animal model to be used for the derivation of the ADI and the AOEL (Acceptable Operator Exposure Level)?"

The initial regulatory position was based on the dog NOAEL, which was the lowest NOAEL in a series of 90 days toxicity studies. However, allometric scaling of various kinetic parameters (renal clearance, plasma half-life) for various species (including mouse, rat, dog, pig, calf, human) showed that the dog can be considered as a clear outlier for compounds such as 2,4-D and MCPA (4-chloro-2-methylphenoxyacetic acid). Allometric scaling is used to see whether species differences in key kinetic

[3] TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION. The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedure and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

parameters such as clearance and half-life are logic based on the differences in body weight and general energy consumption (basic metabolic rate) or not. If not, then the differences observed are probably due to species specificities that have no link with size, body surface area or basic metabolic rate. In the case of 2,4-D and MCPA, the dog exhibits a renal clearance lower and plasma half-life longer than expected based on allometric scaling. After allometric scaling of these parameters, they were found similar for all species, except the dog. At oral dosing of 5 mg/kg, in dogs, the plasma half-life for 2,4-D and MCPA were approximately 100 h and 63 h, respectively. This is substantially longer than in rat (about 1 and 6 h, respectively) or in humans (12 and 11 h, respectively). This longer half-life, and slower elimination (clearance) in the dog, results in substantially higher body burdens of these organic acids, at comparable doses, relative to other species (Timchalk, 2004). The low clearance prompted research into the mechanistic reasoning behind the dog specificity. This resulted in the finding that the dog has a reduced capacity of urinary excretion of weak organic acids in general, of which 2,4-D is an example. So this effect was found not to be compound specific but chemical group specific, and it was concluded that mice and rats appeared to be the preferable species to be used as starting point for human risk characterisation (SCP, 2001). In other words, the dog was 'dismissed' as a relevant model for man for the hazard and risk characterisation of 2,4-D. These data are consistent with the increased sensitivity of dogs (i.e. lower NOAEL as mentioned above).

Lessons learned

This case illustrates the importance to consider at least performing a limited comparative species kinetic analysis in order to determine the most relevant species to be used in toxicity studies (Timchalk, 2004). The lack of relevance of the dog for assessing human health risk for 2,4-D raises questions as to whether future toxicity studies should still be conducted with the dog for other similar compounds, i.e. other phenoxy acetic acids.

4. Discussion five case studies

This paper presents cases where information on one or more ADME parameters or on AUC estimations, as a more integrated TK parameter, was or could have been helpful for a proper human risk characterisation.

4.1. Route-to-route extrapolation

The general lesson learnt from case study 1 (*PPD* as hair dye) is that there may be a concrete need for adequate information on human *and* animal kinetics in order to conclude whether a proposed use is safe. In this case actual information on the diurnal systemic exposure in man and animal expressed as AUC appeared necessary for an adequate risk characterisation. A conventional default approach that does not take into account route-specific systemic exposure (bioavailability) may err on the wrong (unsafe) side. One general reason for this is that for route-to-route extrapolation, no general assessment factors can be given (De Raat et al., 1997; Rennen et al., 2004). Differences in bioavailability between one route (in one particular species, for cosmetics often the oral route in the animal toxicity studies) and the other route (in the human species, for cosmetics often the dermal route) are in general not assumed to be covered by the general and overall assessment factor or required MoS of 100. The minimal MoS of 100 resembles interspecies differences and intraspecies variation in toxicokinetics and toxicodynamics (assuming similar conditions, exposures otherwise). Route-specificities are actually phenomena not covered explicitly in most risk assessment frameworks. This concerns quantitative differences in levels of absorption (relative in percentage) or better, differences in the rate of absorption per square centimetre, but also route-specific metabolic breakdown (first-pass metabolism).

In case the AUCs of the parent compound *PPD* in rat and man would not have been available, the use of *PPD* in hair dyes would have appeared safe in the 2012 evaluation (MoS of 200 where the minimally required MoS is 100). Whereas by calculating the MoS on the AUCs in the rat and in the human (as internal exposure estimates), the MoS is lower than the minimal MoS and the outcome is considered borderline case. Measuring AUCs of the hypothesised toxic moiety (parent compound or a metabolite) in experimental animals as well as in exposed human volunteers has great advantages. By taking these into account in a TK-based risk characterisation, route-specific differences in absorption and first-pass metabolism are automatically taken into account which result in more relevant risk estimates. In this respect, this first example more or less sets the stage how an optimal set of TK data for a proper human risk characterisation should look like.

The second and third example, UV filters *camphor benzalkonium methosulphate* (SCCP, 2008) and *bis*(*butylbenzoate*) *diaminotriazine aminopropyltrisiloxane* (SCCS, 2010b) showed that if route-to-route extrapolation fails, the safety assessment for a cosmetic ingredient is not feasible. Oral bioavailability in the oral toxicity study used to derive a MoS (oral AUC) was assessed to be low or even very low. However, the information was regarded insufficient to conclude on a quantitative level. Adequate delineation of the oral absorption or bioavailability in the key animal effect study is crucial for route-to-route extrapolation as the NOAEL has to be converted to a systemic or internal NAEL in order to establish an internal basis for route-to-route extrapolation. In case that oral bioavailability turns out to be very low and acute and/or subacute (28-days studies) fail to exhibit any adverse effects, one can even debate on the relevance of any (further) repeated dose oral toxicity testing for these chemicals.

The first example as discussed above (*PPD*) nicely illustrates how information on the AUC in the oral toxicity study (in combination with human systemic exposure) equipped SCCS with the necessary information in order to conclude that the use of *PPD* as hair dye ingredient under the foreseen circumstances is of no concern.

The toxicity studies as available for the cosmetics framework are mainly performed via the oral route and no route-specific (i.e. dermal) toxicity studies are available. Therefore, route-specific absorption data are needed to perform a proper risk characterisation for human exposure to cosmetic ingredients as human exposure is via the dermal route. Also within other risk assessment frameworks, this phenomenon of route-to-route extrapolation is recognized. In the framework of occupational exposure risk assessment (as performed by the EU Scientific Committee on Occupational Exposure Limits (SCOEL)), route-specific toxicity data (i.e. via the inhalation route) are preferred as well. However, as these data are often not available, route-specific absorption data are needed in order to properly use oral toxicity data for risk characterization for the human inhalation exposure.

Another interesting and more general phenomenon that pops up from the cases from the cosmetics arena is the following. Low dermal absorption is a favourable property for chemicals intended to be used as UV-filters. Substantial dermal absorption might induce effects in humans whereas the intended action (protection against UV-light) does not require skin penetration. For absorption via the oral route in the animal study it is the other way around. Route-to-route (oral-to-dermal or oral-to-inhalation) extrapolation is often a necessary part of the risk characterisation based on an animal oral toxicity study. This means, it is less relevant what the effect (size) is of the orally administered dose. It is more relevant how large or how little the systemic or

Table 3
Effect of differences in oral absorption (scenario A and B) on the systemic point of departure (internal NAEL) of a hypothetical chemical.

Scenario	Human exposure (mg/kg bw/d)	Animal external oral NOAEL (mg/kg bw/d)	Animal oral absorption (%)	Animal internal NAEL (mg/kg bw/d)	MoS
A	0.1	10	100	10	100
B	0.1	10	5	0.5	5

internal dose was that caused that effect. The less the oral absorption, the more potent a chemical is at the POD. With similar external oral NOAELs for two chemicals, the chemical with the lower oral absorption is inherently more toxic from an internal, systemic point of view. This is directly obvious when PODs are converted to internal PODs such as internal NAELs or internal BMDLs.[4] Just to give a numerical and theoretical example, suppose a chemical exhibiting a NOAEL of e.g. 10 mg/kg bw/d, has completely different oral absorption /bioavailability in different scenarios (see Table 3). In scenario A, the chemical may have been completely bioavailable (100% absorption) in the chronic animal study underlying the NOAEL whereas oral bioavailability in scenario B may have been only 5%. In practice, this means that the intrinsic (internal) toxicity in scenario B is more severe (factor of 20). In scenario A, the value of the internal NAEL is the same as the value of the external NOAEL, i.e. 10 mg/kg bw/d, whereas the internal NAEL for scenario B is 0.5 mg/kg bw/d. Lower oral absorption implies a lower internal NAEL and thus inherently more potency. When comparing the external oral NOAEL to the human internal dermal (or inhalatory) SED, assuming full oral bioavailability in the oral toxicity study is thus far from a conservative approach.

In addition, care should be taken that if expressed as percentage, human dermal absorption should be studied under relevant exposure conditions. Temperature, humidity and anatomical side of the skin are important. Additionally, the surface area dose is important. Relative absorption tends to increase when lowering the surface area dose (Buist et al., 2003, 2009). Even when standardised such as for SCCS evaluation, the standardised approach may not mimic that actual situation. For one chemical, significant decrease in relative absorption starts at lower surface area dose than for another chemical (Buist et al., 2009).

From the case studies illustrated above it can be concluded that the safety assessment procedure can be accelerated and adjusted when both qualitative (parent compound and/or [pre-systemic/first-pass] metabolites) and quantitative (their levels) information on internal exposure in the animal toxicity study as well as under human exposure conditions is generated in due time. In some cases, route-specific and species-specific absorption data are sufficient in order to establish safe use of a cosmetic ingredient. In other cases, more detailed information on internal exposure in humans and experimental animals such as the AUC of the plasma concentration-time profile is necessary in order to conclude on safe use. The best chances for a relevant and accurate risk characterisation are available when AUCs are available for the animal toxicity study and the human exposure conditions.

4.2. Species selection

The fourth case on *β-carotene* (SCF, 2000; ANS, 2012) illustrates how subconscious neglect of important interspecies differences in metabolism can result in erroneous human exposure limits such as an ADI. Pre-systemic gut microflora-dependent clearance of a chemical can result in crucial interspecies differences in systemic exposure, qualitatively as well as quantitatively. In the case study, human internal exposure is mostly to *β-carotene*, whereas in the rodent studies, systemic exposure is mostly if not completely to vitamin A. One suggestion in this respect is to consider *in vitro* testing of qualitative and quantitative pre-systemic metabolism in various species for regulatory purposes of food additives. Another suggestion is to consider clinical studies under controlled circumstances in order to establish human internal exposure qualitatively and quantitatively, analogous to phase I clinical trials for pharmaceuticals as a prerequisite or as part of a re-evaluation program for food additives. Part of the latter could be application of the relatively new methodology of human microdosing. Microdosing is a technique for studying the kinetic behaviour of chemicals in humans through the administration a labelled-chemical in doses so low that it is not intended to produce any pharmacologic effect when administered to humans and therefore is also unlikely to cause an adverse reaction. Human microdosing has been a methodology used as so-called Phase 0 clinical trial in drug development since a few years. The annual number of publications regarding kinetic microdosing is clearly increasing over the last 10 years (Rowland, 2012). The load of preclinical safety assessment that is required before employing microdosing is interestingly low. Repeated-dose toxicity testing in a rodent and a non-rodent species for at least 14 days is generally sufficient for a clinical trial (of microdosing) up to 2 weeks in duration (ICH, 2009).

The fifth example on *2,4-D* (SCP, 2001) as a member of the chemical group of phenoxyacetic acid herbicides nicely shows how relevant species-differences at the other far end of the exposure – intake – uptake – distribution – metabolism – excretion continuum can be for a proper risk characterisation. Species-specificity in the renal clearance of phenoxy-acetic acids in the dog dismisses the dog as a relevant animal model to test systemic repeated dose toxicity. Inclusion of the 90 days dog study for human risk characterisation would have resulted in unnecessary low human exposure limits and possible denial of intended use in certain crops. By investigating the species-specific substrate specificity of renal anion transporter proteins upfront, irrelevant and ethically unacceptable testing in the wrong animal model could be avoided. There is a general feeling that within the EU, it is quite difficult to waive a dog study as a second species for 90-days toxicity testing in the regulatory framework of plant protection products. Upfront discussions between regulators and industry, i.e. before performing regulatory required repeated dose testing with the dog as a secondary species or even any species at all on the (non)relevance of using such animal model are important. This could avoid waste of animals, time and resources and guarantee that the dossier with animal toxicity data submitted for registration purposes contains the most relevant information for human risk characterisation.

5. General discussion

Based on the five case-studies presented in this paper, it is concluded that human health effect characterisation and subsequent risk characterisation for chemical exposure can be improved in many cases by taking into account more and better information mainly on absorption (cosmetics case-studies 1–3), metabolism (*β-carotene*), excretion (*2,4-D*) and/or more integrated figures of a chemicals

[4] Defined as external POD multiplied by the percentage absorption: e.g. external no-observed-adverse-effect leverl (NOAEL) is converted to an internal no-adverse-effect level (NAEL).

toxicokinetic such as AUCs. The level of complexity and intensity of the TK information needed is dependent on the development phase. The issue itself probably holds for all stages in the development and registration of a chemical under any legislative framework, from screening level testing to preliminary hazard and risk characterisation, regulatory testing (i.e. number of toxicity studies required by regulations) and regulatory risk characterisation.

Preferably, a testing strategy should start by investigating some basic toxicokinetics of the chemical. Even screening level information on species specificity (man versus rat, mouse, rabbit), exposure scenario versus toxicity testing scenario (vehicle, concentrations in matrix, skin surface area dose etcetera), route specificity (oral versus dermal versus inhalation) or bioavailability (sum of absorption and route-specific metabolism) can be very helpful in steering (further) toxicity testing.

Ideally the aim should be to include the issue of 'upfront or parallel investigations' of ADME/TK in protocols. Eventually, the requirement to address the issue as such could be taken up in regulatory requirements and Test Guidelines for studying human health effects. This is actually preferred over the general current strategy which is relying on separate studies on ADME/TK, i.e. full-blown toxicokinetic studies (e.g. according to OECD Test Guideline 417 'Toxicokinetics' or OECD TG 428 'Skin absorption: *In vitro* method'). This latter strategy includes the risk of being performed under different test conditions (route, dose, species/strain etc.) as compared to those of the critical health effect study. More specific, information on the internal exposure (i.e. AUC, C_{max}) would be supportive to understand the observed health effects in a mode of action framework and to link the external dose to the internal exposure. Preferably and similar to determination of safety margins between pharmacologically desirable plasma levels and toxic plasma levels (Smith et al., 1990), determination of Margins of Safety between human exposure and animal points of departure (BMDL or NOAEL), should be based on AUC and C_{max} values in man and experimental animal. The hypothesized mode of action (MOA) should drive the choice for AUC or C_{max}.

Some progress is noted in the field of pesticide risk assessment, though, at least in the hazard testing domain of the risk assessment framework. About half a decade ago, a strategy was presented to assess systemic diurnal exposure in animal toxicity studies (24 h AUC expressed as mg/kg bw/d) by utilizing a minimal number of blood samples in subchronic diet and drinking water studies. Determination of parameters such as blood/plasma half-life and internal exposure (i.e. AUC) in the early stages of testing provided critical information to improve the appropriate design of other longer-term toxicity studies (Saghir et al., 2006). As a follow-up, very recently a procedure was described for the direct integration of TK in regulatory toxicity testing of agrochemicals, its main goals being to improve toxicological study design and to reduce animal use (Saghir et al., 2012). The procedure included the establishment of kinetic behaviour of the test material, including computer modelling to assess the optimal blood sampling time points already in an early stage of the testing programme (Saghir et al., 2012). It presents a practical example of the recommendations published earlier in Barton et al. (2006). TK was implemented in all in-house dietary guideline toxicity studies to assess the diurnal systemic dose. The integrated TK data obtained across toxicity studies (without additional/satellite animals) were critical to understanding differences in response across doses, species, strains, sexes, and life stages (Saghir et al., 2012).

6. Conclusion

Conclusively, the case studies presented here illustrate the increased potential that data on toxicokinetics could have. Both animal and human kinetic information can be useful for the design of animal toxicity studies. Moreover, this information is often pivotal for an accurate risk characterisation. Based on mode of action considerations, determinant in all cases presented is probably AUCs in man and animals. In general, by using AUC or C_{max}, species-, route- and exposure scenario-dependent differences in (time-dependent) systemic exposure are integrated to the largest extent feasible.

Increased implementation of toxicokinetics sampling in all stages of toxicity testing could provide significant improvements in terms of efficiency, relevance, reliability, time constraints and budget. Moreover, it could help to refine (less high dose testing) and reduce (less irrelevant testing) the number of animals necessary in order to guarantee safe use of chemicals. As such, animal and human kinetics are regarded a crucial building block in the construction of a more human exposure-based risk assessment paradigm. Such a paradigm will help to maximise human relevance while minimising irrelevant animal testing.

Conflict of interest statement

None.

Acknowledgments

The authors acknowledge Peter Bos and Jacqueline van Engelen for critical comments during the final stage of the manuscript and the following experts who provided us valuable information on the testing and risk characterisation procedures as well as case studies within the various frameworks: Marloes Busschers and Angelique Welten (Ctgb), Jacqueline van Engelen, Rolaf van Leeuwen, Wim Mennes, Marja Pronk, Trijntje van der Velde-Koerts and Gerrit Wolterink (RIVM) and Ruud Woutersen (TNO). The Netherlands Ministry of Public Health, Welfare and Sports is acknowledged for providing funding the underlying research.

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