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ROLE OF PHARMACOKINETICS IN SAFETY EVALUATION AND REGULATORY CONSIDERATIONS*

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INTRODUCTION

The Food and Drug Administration (FDA) regulates the efficacy and safety of drugs and the safety of food and color additives as part of its mandate to protect the public health under the Federal Food and Drug Act. Drugs are pharmacologically active agents that are regulated on a benefit/risk basis which considers their ability to produce both specific therapeutic effects and minimal undesirable side effects. On the other hand, food and color additives are regulated on a risk-only basis. Both drugs and food additives are precleared by the FDA prior to approval for marketing. Demonstrations of drug efficacy and safety primarily depend upon data generated in human trials at intended human therapeutic use levels. Animal studies are used primarily in the early stages of drug development and in the initial stages of drug regulation. However, demonstrations of food safety are ordinarily totally dependent upon the use of human surrogates; animals, usually rodents, are typically exposed at dose levels several orders of magnitude above expected human exposure levels in both acute and chronic toxicity studies. Extrapolation of

these data is therefore necessary not only from high to low dose within a species, but also from animal to human. Pharmacokinetic data (absorption, distribution, metabolism, excretion) describe a chemical's disposition and allow for accurate determination of dose-response toxicity relationships within a species. Pharmacokinetic (PK) data can help make the cross-species comparison of the relative activity of specific metabolic pathways. Pharmacokinetic data can, therefore, help improve the scientific basis of risk extrapolation and thus increase confidence in the overall risk-assessment process. The FDA recognizes that PK data can play an important role in the design and evaluation of toxicological studies and encourages the submission of such mechanistic data.

BASIS FOR THE APPROVAL OF NEW DRUG COMPOUNDS

Before a drug becomes widely available for use in humans, it first must be approved by the Center for Drug Evaluation and Research (CDER) at the FDA. Drugs are compounds that are known to be pharmacologically active and are purposefully administered in order to produce some therapeutic effect. But, because drugs may cause more than one physiological effect, it is necessary to determine the actual effects that occur and how severe they are. Therefore, the regulation of drugs considers both their efficacy and also whether or not they produce serious, unwanted (side) effects. A drug's safety is in fact defined on the basis of the capacity to minimize these side effects and keep them within acceptable bounds. Both animal and human studies are used for the evaluation of a new drug compounds. Approval is granted when the drug has been determined to be both safe and efficacious when used in the manner intended.

ROLE AND IMPACT OF PHARMACOKINETICS IN THE APPROVAL OF NEW DRUG COMPOUNDS

Animal Studies

The approval process for a new drug compound begins wth the submission of an Investigational New Drug (IND) application. The IND application contains the results of acute toxicity and pharmacology tests conducted in laboratory animals and also includes protocols for the conduct of human (clinical) studies. Approval of the IND is based, in part, on the results of animal toxicity tests, which are conducted in order to determine if "it is reasonably safe to conduct the proposed clinical investigations" (1), and, in greater degree, on the adequacy of the design of human clinical trials.

Acute and sub-chronic animal studies are designed to determine the safety of a new drug compound by characterizing its disposition and physiological

effects, both therapeutic and toxic. Specifically of interest are the dose range over which the pharmacologically desired effect occurs, the dose level at which toxic side effects are induced, the scope of toxic effects from gross physical changes (dehydration, lassitude) to biochemical and physiological effects (renal damage, enzyme changes), and the effect of a multiple dose regimen (accumulation, enzyme induction). Correct design and interpretation of animal toxicity data is necessary to ensure that human trials will be conducted safely. Determination of PK parameters, such as rates of elimination and absorption, bioavailability, maximal blood concentration (C_{max}), time to C_{max} (t_{max}), area under the concentration curve (AUC), renal, metabolic and/or total body clearance, provides a quantitative description of a drug's disposition profile that can be used to compare profile differences across species.

Pharmacokinetic data from single exposures can be used to help determine appropriate dosing regimens for sub-chronic and chronic studies. Determination of half-life following oral administration may indicate that once-per-day gavaging of drug may produce high concentrations for short periods of time, whereas administration via the feed may produce prolonged absorption and low concentrations for extended periods. Selection of the appropriate regimen for use in animals will depend on the proposed human-dosing regimen and route of administration.

Comparison of PK parameters obtained following multiple doses to those obtained following single doses would allow one to determine if the drug induced or inhibited its own metabolism. When chronic studies are conducted, it is assumed that the animal's daily exposure will be the same throughout the course of the study; this may not be the case. If an animal is able to increase its rate of metabolism of a drug, then exposure of that animal to the parent drug will be less than the researcher has predicted and exposure to metabolites will be increased. Depending on which compound, parent or metabolite, is the effective agent, the effects observed, therapeutic or toxic, may be greater or less than predicted.

Although blood (serum and plasma) and urine are the most commonly sampled body fluids, determination of tissue disposition can also provide important clues about drug disposition. Determining if drug is concentrated in specific organs or tissues would provide an indication of which tissues might be targets of toxicity; higher concentrations would increase the possibility that these tissues suffer a greater toxic insult. Also, accumulation in a noneliminating tissue may provide a depot from which drug may be released and therefore drug may remain in the body for prolonged periods even though concentrations in body fluids and other tissues are too low to measure. Thus, accumulation in a specific tissue may become the limiting factor when designing multiple-dosing regimens.

Correlating observed toxicity with appropriate PK parameters may allow

the investigator to more accurately interpret toxicity tests data and even predict at what dose toxicity should occur and help understand the mechanism responsible for the effect. For example, a steep dose-response curve may be caused by a nonlinear rate of elimination; saturation of an elimination process can result in disproportionate increases in plasma concentrations (C_{max}, AUC) and may produce a higher level of toxicity than would be predicted by linear extrapolation of responses seen at lower doses. Conversely, if the degree of toxicity remains the same with increasing dose (a plateau region), then saturation of absorption processes may be the cause; when saturation of absorption occurs, the total amount of drug absorbed, and toxicity seen, following two different doses may be the same, even though the amount of drug administered is different.

Comparison of PK data obtained from different animals given the same dose will indicate the degree of population variability in drug disposition; it may also indicate the source of the variability. For example, comparison of the total amount of drug excreted unchanged into the urine can indicate if metabolism or excretion of the drug is extremely variable. If the total amount of drug excreted unchanged is very different then this would indicate that the amount of drug metabolized was variable. If the total amount of drug excreted unchanged was the same but the rate at which it was excreted was different, then this would indicate variability in the excretion process. If the population variability for a drug is high in laboratory animals, usually homogeneous and inbred populations, then even larger variations in response would be expected for humans.

CDER requires that animal studies be conducted in at least two species of laboratory animals, one rodent and one nonrodent. The results from each species must then be compared in order to determine what similarities or differences exist. Frequently, the same dose of drug (mg/kg body weight) is given to two species and the observed responses are different. Pharmacokinetic analysis may reveal that the concentration versus time curves in both species are similar, and therefore, the intrinsic sensitivity or degree of response of the species is probably different. Conversely, it may be found that the bioavailability of the drug is significantly lower for one species, resulting in much lower concentrations of drug in the body. If the administration of higher doses produces the same blood concentrations of the parent drug and now produces the same response as seen in the other species, this would indicate that the intrinsic sensitivity of the two species is the same but that they metabolize the drug differently.

Human Clinical Trials

Human studies are usually conducted in three phases. Phase 1 studies, are "designed to determine the metabolism and pharmacologic actions of the drug

in humans, the side effects associated with increasing doses and early evidence of effectiveness" (2). Phase 2 studies are controlled clinical studies usually conducted in a small number of patients with the disease for which the drug is intended. Demonstration that the drug is effective should be made at this time. Phase 3 studies include a broad-based clinical trial, usually involving hundreds of patients as well as specific control groups. The clinical trial is designed to provide proof of safety and efficacy of the new drug in a larger population.

Determination of PK parameters and better characterization of drug disposition in animals may allow the investigator to design safer human studies. In Phase 1 studies, results from animal studies may be used to adjust the intervals between dose levels in dose-escalation studies (3). For example, if the ratio between pharmacologically active and toxic doses is small or there is an abrupt increase in the dose-response curve in animals, then initial dose-escalation studies should use smaller increases between doses. Also, determination of target organs or depot sites in animals would allow the investigator to monitor clinical signs that indicate altered function or overt toxicity at these sites in humans.

Determination of PK parameters, in humans, as part of Phase 1 trials would again allow for a more complete and detailed description of the dose-response relationship and provide a basis for comparison of data to animals. Comparison of the dose-response curves can indicate if human response is similar to that of the animals studied and whether or not humans are more or less tolerant to side effects. This would enable the investigator to determine how representative the animal model was in predicting human safety.

In Phase 2 trials, PK data can also provide an important role in the determination of the efficacy of a new drug. Different responses following administration of a drug may be due to differences in the ability of the patient to respond or to differences in drug disposition. Comparison of PK parameters from individual patients would enable the clinician to determine if interindividual variation in drug disposition is very high. The efficacy of a new drug may seem independent of administered dose but be very well correlated to achieved blood concentrations and PK parameters such as AUC, t_{max} and C_{max}.

In developing an effective therapeutic dosing regimen, PK data can be used to statistically compare drug disposition following different routes of administration or administration of different drug formulations. In many cases, the toxic effects of a drug may be avoided or reduced by altering the dosage regimen or dose formulation. Smaller doses given more often minimize the swing between peak and trough concentrations and higher concentrations that produce toxicity may be avoided. Also, dosages may be formulated to produce a prolonged absorption phase where peak concentrations are reduced but therapeutic concentrations are retained.

The approval of a new drug compound is based on the decision of whether the drug is both safe and effective. This decision is based on the interpretation of data obtained in animal and human studies, which are submitted to CDER as part of a New Drug Application (NDA). By providing a more complete description of the disposition of a new drug compound, pharmacokinetic data can be used to support conclusions drawn from toxicity and efficacy tests by providing explanations for why similarities or differences occur. These comparisons can increase the confidence that toxicity and efficacy data are being correctly interpreted, and the number of animals needed for toxicity tests and patients needed for determination of efficacy in clinical trials may then be reduced.

Generic Drug Formulations

One area where PK data has had a big impact is in the approval of new drug formulations, more commonly known as generics. If the new formulation is intended to be used interchangeably for the same therapeutic effect as the originally approved formulation, then approval may be granted if it is shown that the bioavailabilities of the two formulations are equivalent. Formally, bioavailability is defined as "the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed from a drug product and becomes available at the site of drug action" (4). Bioavailability can be easily characterized and quantified by the determination of several PK parameters: AUC (for at least three elimination half-lives), C_{max}, t_{max}, total amount excreted (either parent or metabolite) and rate of excretion. Statistical comparison of these parameters is sufficient, in many cases, to show equal bioavailabilities or bioequivalence and thus obtain approval.

The demonstration of bioequivalence also precludes the need for the submission of a completely new NDA. An abbreviated NDA may be filed in which reports of nonclinical and clinical studies, except those pertaining to the bioavailability of the new formulation, are omitted (5). This translates into an effective saving of time and money for both industry and the FDA.

BASIS FOR REGULATORY SAFETY REQUIREMENTS OF FOOD AND COLOR ADDITIVES

Like drugs, food and color additives must be approved (by the Center for Food Safety and Applied Nutrition (CFSAN) at the FDA) before they are allowed to be marketed. Evaluation of food and color additives is usually based on data obtained from a variety of animal studies in which the animals are typically given doses that are orders of magnitude higher than expected human exposures. Human data are not usually required and are normally unavailable. Therefore, in order to estimate human risk following exposure, it

is necessary to extrapolate dose-response data from animals to man, from high-to-low dose and, sometimes, from one route of administration to another. The goal of risk assessment is to set reasonable food and color additive tolerance levels for exposures in order to assure safe levels for humans.

ROLE AND IMPACT OF PHARMACOKINETIC DATA ON THE REGULATION OF FOOD AND COLOR ADDITIVES

Pharmacokinetic principles and specific PK data can improve the design and interpretation of animal studies and therefore enhance the relevance of animal studies for estimating human response. By improving our ability to extrapolate animal data across species, including man, from high-to-low dose and from one route of exposure to another, we also improve the dose-response estimation component of the overall quantitative risk assessment process. Better estimates of effective dose, scaling and safety factors, and of the influence of nonlinear kinetics on compound disposition increase confidence that human risk is being accurately assessed. Physiologically based pharmacokinetic models, a relatively recent development, offer a powerful tool for estimating, analyzing, and comparing PK parameters within and across species.

Design and Interpretation of Animal Studies

CHRONIC STUDIES The design and interpretation of results of chronic bioassays are of particular importance in the regulatory process. As with drugs, pharmacokinetics can improve our ability to design studies and interpret data. Incomplete dose-response data can lead to increased estimates of human risk and lower estimates of acceptable exposure.

Traditionally, toxicity data have been correlated to administered dose. Measurement of blood concentrations followed by pharmacokinetic analysis has shown that, in many cases, administered dose is not an accurate measure of exposure. Substitution of blood levels or measures of effective dose for administered dose may improve predictions of toxicity when extrapolating data (6).

Because of the small number of animals (50–100/dose) that can practically be used in chronic studies, cancer would never be detected if animals were dosed at expected human exposure levels. Thus, animal doses in chronic studies are typically set at large multiples of likely human exposure, with the highest dose being the maximum tolerated dose (MTD) (7, 8). The lowest dose is usually one at which there is little or no observed toxicity and yet is still a significant multiple, usually several orders of magnitude over expected human exposure levels.

Appropriate selection of the MTD for chronic bioassays is extremely important. The MTD is supposed to be the highest dose that can be tolerated by the animal before such significant qualitative and quantitative organism effects are induced that the test results are deemed uninterpretable for animal or human safety. There are two inherent difficulties in determining the MTD: first, it is needed prior to the long-term study and can only be estimated from an earlier, necessarily shorter, usually 90-day, study. Second, there exists no really adequate definition of a measurable endpoint. Current NTP practice includes consideration of several different factors in the determination of an MTD (9). These factors include hematology, weight loss, organ function, histopathology, hormonal and immune disturbances, clinical chemistry, other toxic signs, and consideration of pharmacokinetic data.

Because chronic studies for food and color additives typically require only three or four doses (10), it is important that the doses be appropriately spaced so that the dose-response curve may be adequately defined. The number and spacing of dose levels below the MTD can be improved by using PK data from acute and subchronic studies to estimate where blood or urine levels of parent compound or major metabolites saturate. Doses below the MTD could be selected to cover both the linear and nonlinear portions of the disposition curve. Determining the response of an animal at dose levels above and below saturating processes is important because toxicity may be related to the saturation of some detoxification pathway, metabolic pathway, or route of excretion.

Pharmacokinetic data may improve the interpretation of a chronic bioassay by identifying metabolic and disposition pathways that may be saturated at the MTD. If the highest animal dose were to cause saturation of a detoxification pathway, then a case might be made that the observed compound-induced cancer was solely the byproduct of toxicities induced by excessive accumulation of the toxicant due to pathway saturation and that cancers would effectively never occur at lower doses. For example, the dose-response curves of some genotoxic compounds seem to exhibit a threshold effect. In particular, it has been proposed that, at low doses, DNA-repair mechanisms are able to cleave adducts and significantly reduce the damage caused by the presence of the carcinogen. At higher doses, when the repair mechanism is saturated, the number of adducts produced becomes proportional to dose and the presence of tumors increases proportionately (11).

TARGET ORGAN CONCENTRATIONS Identification of all organs that may be affected by a food or color additive is also important for accurate interpretation of bioassay data and complete hazard identification. Target-organ toxicity is often correlated to tissue distribution of test chemical or its metabolites. Disposition data can indicate which tissues have high concentrations of the

chemical and may therefore be at greater risk. Tests that measure changes in the function of that organ may detect more subtle signs of toxicity than seen by gross observation or pathological examination. For example, it is well known that changes in in vitro tests of enzyme activity (e.g. cytochrome P-450, glutathione-S-transferase, glucuronide conjugation) in liver homogenates can reflect changes in in vivo liver function.

Disposition data may also indicate other types of toxicity tests that may not be needed or that may have to be redesigned. For example, if the compound does not cross the placenta in significant amounts then, in utero exposure tests, such as used for teratology tests, may not be necessary. A study designed to test the effect on postnatal development may utilize exposure through mother's milk. But, if the compound is not measurably secreted into mother's milk, then concentrations of exposure to young during feeding may be too low to induce toxicity, resulting in a false negative result.

SELECTION OF ANIMAL MODEL Testing of food-supply chemical compounds for acute and chronic effects must be done in animals as human surrogates. The determination of the most appropriate animal to be used in toxicity testing has always been a problem. It would be desirable if test animals were selected so that they were similar to humans in both their intrinsic sensitivity and pharmacokinetic handling of the test compound. However, more often than not, the selection of an animal model is based on considerations of cost, size and availability of the animal, housing requirements and lifespan. In the absence of knowledge of PK and metabolism data, animal selection has tended toward the use of animal-test species that are most sensitive and/or for which there is an availability of historical controls. For example, the hamster is generally used for teratology testing of nitriles (12) and the National Toxicology Program (NTP) has built up a large historical experience with F344 rats and B6C3F1 mice used for chronic cancer bioassays (13).

Pharmacokinetics is a tool that can be used to further our understanding of the biology of laboratory animals and improve our interpretation of toxicity data. For example, dogs and cats both show significant but different toxic effects when administered ethylene glycol. For dogs, the lethal dose is 4.5 mg/kg body weight (BW) and they usually die from uranemia or acidosis. For cats, the lethal dose is 1.5 mg/kg BW and they usually die from depressant effects or acidosis. Ethylene glycol is itself toxic and it is also metabolized through a series of acids to oxalic acid, which can combine with calcium and precipitate in the kidney. Dogs are more resistant than cats, i.e. the toxic dose is higher, because dogs effectively metabolize ethylene glycol, reducing their exposure to parent compound. However, they do ultimately suffer from the effects produced by the formation of the acids. Because cats slowly metabo-

lize ethylene glycol, the primary toxic effects are the result of prolonged cumulative steady-state exposure to unchanged ethylene glycol levels (14).

By understanding the source of species similarities and differences in disposition, we can expand our knowledge of the factors that contribute to differences in intrinsic sensitivity and be better able to extrapolate those results to man. If human data do become available, then PK data could be used to refine the applicability of animal tests by permitting the determination of whether differences in toxicity are the result of differences in PK or intrinsic sensitivity.

Dose-Response Estimation of Human Risk

One regulatory goal of food safety evaluation is to set exposure levels that correspond to acceptable daily intake (ADI) levels for humans. In principle, this requires qualitative as well as quantitative information on dose-response toxicity. Noncarcinogenic responses are typically nonlinear in observable ranges and assumed to be thresholdable (no risk at all) at lower doses. Carcinogenic responses are sometimes nonlinear in observable ranges but are typically assumed to be linear at low dose. That is, some risk is assumed for all finite doses. In either case, quantitative information on dose-response is critical to establish safe levels of compound use for humans. For carcinogenic endpoints, at least, quantitative risk-estimation tools attempt to provide a reasonable upper bound on likely human risk at lower human-exposure levels. Refinement in several pharmacokinetic areas may potentially contribute to the improvement of classical human-risk estimates. These include (*a*) the use of alternative measures of dose, (*b*) the selection of more appropriate interspecies scaling factors, (*c*) the consideration of nonlinear kinetic effects in high-to-lose dose extrapolation, and (*d*) the development of physiologically based pharmacokinetic models.

ALTERNATIVE MEASURES OF DOSE Perhaps the most important single role that PK data and models can play in the improvement of human dose-response risk estimation is to define truer measures of animal exposure to the test compound that can be substituted for administered dose in dose-response determinations. A truer measure of exposure is frequently called the effective dose and it is assumed that this measure is more biologically related to the observed toxic response than is administered dose. Effective doses may be whole body measures, such as AUC of the blood concentration or amount excreted into the urine. An effective dose may also be specific for some tissue or organ, such as peak concentration in the kidney, number of subcellular DNA adducts in an organ, or, more typically, AUC of the target-organ concentration. Although blood levels may be more conveniently measured and can usually be determined before target organs are identified, they are one

further step removed from the ultimatc biological response of interest than is target-tissue concentration. Though cellular and subcellular measures may be closer to the eventual biological response, they are harder to measure, to mechanistically validate, and to correlate with the ultimate toxic effect.

As an example of the quantitative use of PK information for selection and validation of an effective dose measure, consider the case of dichloromethane (DCM) (15). This compound was observed to cause both liver and lung tumors in chronic animal cancer bioassays. Administered dose levels of DCM parallel liver blood levels of DCM, which, in turn, roughly parallel tumor response. Dichloromethane is metabolized essentially by two competing metabolic pathways: the mixed function oxidase (MFO) pathway and the glutathione-S-transferase (GST) pathway. Clearly, either the parent substance or the metabolites from at least one of these metabolic pathways must be functionally related to tumor appearance. Proportionality was also observed between liver tumor response and the AUC of DCM liver blood concentrations and the AUC of liver GST-produced metabolites. Thus both are consistent, without further validation, with being the primary toxicant. At the dose levels of DCM that produced tumors, the MFO pathway was saturated and the AUC of liver MFO-produced metabolites was not proportional to tumor response, suggesting that MFO-produced metabolites are not responsible for tumor formation. Proper choice of the effective dose measure is important in this example because it may change the estimated risk. Use of the GST pathway as the sole carcinogenic pathway would indicate (using initially published information on the GST pathway and assuming breathing rates and cardiac rates were allometrically similar across species) that the human risk is 3–4 times less than when pharmacokinetics are not considered. On the other hand, use of DCM blood liver concentrations gives about 2-fold higher risks (R. N. Brown, FDA/CFSAN, Washington DC, internal memo).

Simple statistical correlation of an effective dose with toxic response is not sufficient in itself to validate the choice of that dose measure. Supporting data on the mechanism of toxicity and the probable relation to the proposed effective dose would increase confidence that a correct choice was made. In the case of DCM, in vitro tests indicated a greater probability that a GST-produced metabolite was the true carcinogen and not DCM itself and therefore it was proposed that risk estimates should focus on the amount of GST metabolites produced (R. H. Reitz, personal communication; M. R. Harris, personal communication).

Current risk-estimation models are primarily efficient procedures for curve fitting the overall toxicity response in the observable response ranges and for providing conservative linear upper bounds on risk in the unobservable response ranges at lower doses. Estimates from current curve-fitting procedures are likely to be biased, i.e. in error, in that they do not reflect the true

underlying pharmacokinetic or pharmacodynamic parameters or response outside the range of experimental animal observability. Ideally, risk models that incorporate the actual biology are desirable to improve risk estimation. If biological models are developed and nonlinearities in PK and pharmacodynamics are taken into account for a given effective dose measure, risk estimates at lower administered doses should be qualitatively and quantitatively improved—overall model bias will be reduced.

When choice of effective dose is uncertain, or there are multiple pathways associated with toxicity, it may be useful to sum weighted probabilities of risk from alternative suspect pathways for purposes of summary risk estimation. These weighted risks may better account for the associated uncertainty in the appropriate measure of effective dose and lead to greater confidence (and perhaps less model bias) in final human-risk estimates than sole reliance upon just one partially validated pathway. With the development of physiologically based pharmacokinetic models and the advent of simulation software, one can now rapidly calculate effective dose estimates for multiple pathways that can be used in these summed or weighted risk estimates.

COMPARATIVE PHARMACOKINETICS The FDA's traditional approach for setting safe levels of noncarcinogenic substances is to establish an effect level in an animal study, then reduce the dose to a "no-observed-effect level" (NOEL) and then apply a safety factor (SF) to this "threshold" value to achieve an acceptable daily intake (ADI) for humans; typically ADI = NOEL/SF = NOEL/100. The factor of 100 was arbitrary but seemed a reasonable approach consistent with experience and with the data available (16). It presumably reflected the observation that humans are generally more sensitive to acute chemical toxicity than laboratory animals given the same unit dose on a mg/kg basis and also that humans are genetically more diverse than the typical inbred strain of laboratory animal. It also reflected concern for the anticipated variations in response from differences in the state of health, type of diet, and other conditions that may vary between humans and animals. Others have attempted, post hoc, to reinterpret this 100-fold safety factor solely as an intrastrain variability factor of 10 and as an interspecies variability factor of 10 (17). But, by either interpretation, the purpose of the safety factor is to allow for uncertainties in our knowledge of the toxic response of a small number of laboratory animals in establishing safe doses for a far larger human population. For compounds tested directly in humans, a factor of 10 rather than 100 is typically used, acknowledging the 10-fold factor assigned to interspecies differences. The safety factor accounts for both pharmacokinetic and pharmacodynamic uncertainties. For example, the human variability in absorption, volume of distribution, renal and hepatic clearances observed for some drugs represent the possible differences in

pharmacokinetic parameters than can exist within the human population (18). In addition to the *intra*species variability, which also exists but perhaps to a lesser extent in laboratory animals, there is the *inter*species problem of ascertaining an effective dose in an animal study and comparing it to the corresponding effective dose in humans. This too is a pharmacokinetic issue and encompasses the problem of size scaling (allometry) and time scaling (physiological timing) between small and large animals. The other possible differences between animal and human responses that remain when concentration-time factors are excluded represent the pharmacodynamic uncertainties.

The traditional ADI is based on administered dose, not on target organ concentration or even blood levels, i.e. AUC. In principle, ADIs could be based on blood levels (AUC) or be derived from them, in this way accounting for both absorption and clearance and thus representing a truer effective dose. But to do this would require corresponding measurements of AUC in humans in order to relate administered dose to AUC in both species. This is at once both the promise and the practical limitation of the application of pharmacokinetic principles to safety evaluation. It is essentially an exercise in comparative toxicology and requires information in both species before much progress can be made. The relationship ADI = NOEL/SF may provide the sponsor of a food additive a level of his product ample enough to satisfy his needs. In this case there is no motivation to conduct additional chemical-pharmacokinetic studies. For other substances, where the ADI has been previously established as above, the sponsor may be able to gain approval for additional uses and raise the ADI after conducting appropriate studies. For example, if it turned out that because of decreased absorption or higher clearance in humans that the AUC-based human NOEL was higher than that based on administered dose, a higher ADI could be approved.

For carcinogenic substances, or at least for that subset of carcinogenic impurities and pesticides currently allowed under the food safety laws, the use of comparative pharmacokinetics is even more important. Current risk-assessment methods are prudently conservative and quite often the acceptable risk-limit tolerance is approached by the sponsor even at quite low levels of carcinogenic impurity. Refinements of the effective dose as the result of the use of more accurate pharmacokinetic parameters can in some cases, as illustrated above by DCM, demonstrate that the actual risk may be lower than the estimated risk based on administered dose. The use of comparative pharmacokinetics in this way may be illustrated generically as follows: Suppose the human (H) risk (R) from a carcinogenic agent can be approximately expressed by the product of dose (D) and potency (P), i.e. $R_H = D_H \times P_H$, where the potency in humans (P_H) is the slope of the dose-response curve, taken to be linear in this illustration. An expression of the same form is also

assumed to be true for animals. The assumption traditionally made when animals (A) are used as human surrogates in cancer studies is that the risks, correctly normalized for both dose and potency are the same: $R_H/(D_H/P_H) = R_A/(D_A/P_A)$. It follows directly that human risk may be expressed in terms of the measured animal risk and the corresponding dose and potency ratios, $R_H = R_A \times (D_H/D_A) \times (P_H/P_A)$. This expression explicitly outlines the risk-estimation process as an exercise in comparative toxicology. What do we know generally about the potency ratios? Cancer is currently regarded as being initiated by a direct attack on the genome and capable of modulation by a variety of cellular, hormonal, tissue or host-sensitive factors. The existing data base from acute and chronic studies that suggests greater sensitivity for humans is not directly applicable to cancer. In addition, the most sensitive animal species is usually selected for carcinogen bioassays and negative bioassays are usually disregarded in the face of positive ones. For these reasons and because the risk extrapolation process (from high to low dose) is conservative, FDA has generally neglected any possible differences in potencies between animals and man. In effect, the potencies are assumed to be equal and the human risk becomes: $R_H = R_A \times (D_H/D_A)$. The dose in this expression is really a dose rate per unit $(BW)^n$, so that human risk becomes: $R_H = R_A \times (D/BW)^n_H/(D/BW)^n_A$.

In individual cases where PK methods can be used to automatically scale dose across species, the need for a generic dose-scaling factor becomes moot. But in the vast majority of cases, the detailed information needed in order to employ PK methods is lacking and the issue of what scaling factor to use becomes important.

Two generic scaling factors are in common use today: one based on normalizing for body mass and the other for body surface area. For example, the FDA and the US Environmental Protection Agency (EPA) typically use different scaling factors to correct for "equivalent toxicological" dose or dose rate across species. Using the FDA's approach, the toxicologically "equivalent" bolus dose in humans and animals could be calculated as $D_H = D_A \times (BW_H/BW_A)$. Using the EPA's surface area approach, the equivalent dose would be $D_H = D_A \times (BW_H/BW_A)^{2/3}$.

Although traditional in toxicology, the average body concentration, obtained by dividing the intake dose by body weight, is a very crude measure of effective concentration. As in the case of noncarcinogenic endpoints, human-risk estimates may be improved by using instead the corresponding steady state blood concentrations (C_{ss}): $R_H = R_A \times (C_{ss}{}^H/C_{ss}{}^A)$. This expression can be expanded to include explicitly the various pharmacokinetic factors that related the blood concentration of the agent more accurately to the effective concentration in the neighborhood of the specific responsive receptor, e.g. bound fraction, appropriate metabolite, fraction of metabolite bound

to the receptor, etc (19). The point we emphasize is that as this pharmacokinetic refinement occurs, it must occur in both the test animal and the human to be useful in comparing the estimate of human risk.

IMPACT OF NONLINEAR KINETICS Because animals must be dosed at levels far above estimated human-exposure levels, extrapolation of the dose-response curve to low levels is the necessary first step in estimating human risk. If there are no nonlinear parameters and no accumulations in any compartments, then an effective dose should be proportional to administered dose and PK-based risk estimates should be identical to classical risk assessments. On the other hand, nonlinearities of accumulation, kinetic and binding saturations, and flow limitations at high doses determine the degree of deflection of effective dose from proportionality to administered dose. Therefore, consideration of how effective dose may be affected by nonlinear kinetics offers great ability to improve upon conventional linear-at-low-dose risk extrapolation models.

Figure 1 shows the equations that describe the AUC for a one-compartment linear model and a one-compartment nonlinear, Michaelis-Menten model. When elimination (or other) mechanisms are no longer independent of concentration, the relationship between AUC and initial dose becomes more complex. When the parent compound is the active agent, one would expect disproportionately higher toxicity with increased dose. Linear extrapolation of the dose-response curve from above the saturation level to lower doses would overestimate toxicity because no account is taken of the increased efficiency of clearance at lower doses. When the metabolite is the active agent, saturation of the biotransformation processes that produce the metabolite will result in disproportionately lower toxicity at high dose and standard linear extrapolation to low dose would underestimate the risk (20, 21).

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS The recent development of physiologically based pharmacokinetic (PBPK) models has provided a new tool that increases our ability to extrapolate from high-to-low dose within a species, estimate exposures by different routes of administration, provide a means for calculating effective doses and extrapolating these values across species. Physiologically based pharmacokinetic models provide the means for quantitatively describing the concentrations of parent compound or metabolites in several physiological organs or combined organ compartments, simultaneously.

Physiologically based pharmacokinetic models differ significantly from classically derived compartmental models in that PBPK "models are elaborated on the basis of the known anatomy and physiology of humans and animals and incorporate physiological, anatomical and physiochemical data"

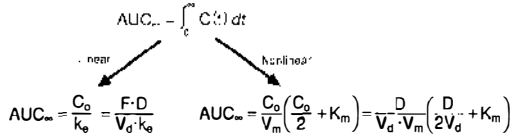

Figure 1 The difference in the determination of the area under the curve between linear and nonlinear metabolic pathways. AUC = time integral under the concentration curve, F = fraction absorbed, D = bolus dose given, V_d = volume of distribution, k_e = elimination rate constant, C_o = apparent concentration (D/V_d), V_M = maximum kinetic velocity of elimination, K_m = concentration of 1/2 maximum kinetic velocity.

(22). Classical models are empirically derived by the curve-fitting of data (by nonlinear regression analysis) and "the compartments and parameters have no obvious relationship to anatomical structure or physiological function" (22). Physiologically based pharmacokinetic models represent the body as a number of compartments connected by the body fluid system (generally taken to be plasma or blood); each compartment can represent one or more tissues or body fluids. Each compartment is usually represented by linear ordinary differential equations but equations that define nonlinear processes, such as Michaelis-Menten kinetics and zero-order absorption, can also be incorporated. All the equations are then solved simultaneously. These models require the determination or estimation of many physiological, anatomical and biochemical parameters; a typical 5–6 compartmental model may have up to 30 or more parameters, depending on the complexity of the equations needed to describe it. Examples of physiological and anatomical parameters are breathing rates, lung capacity and sizes, and blood flow rates of individual tissues. Examples of biochemical parameters are rates of absorption, metabolism and elimination, degree of plasma-protein binding and partition coefficients (22, 23).

Physiologically based pharmacokinetic models are generally validated by comparing what the model predicts to actual data obtained from animals following a single dose of compound by one route of administration. But, the utility of PBPK models lies in their ability to estimate disposition patterns following the administration of a wide range of doses and following different routes of administration. Disposition patterns following any administered dose can easily be determined just by changing the input value. Estimating disposition following different routes of exposure can be done by changing the compartment into which the compound is administered. Intravenous administration can be modeled by introducing the compound into the blood compartment whereas oral administration would be modeled by putting the drug into the intestinal compartment and estimating rates of absorption.

These models can also be used to predict the disposition of drug in any particular compartment (i.e. organ or tissue) by solving the equations that

describe how compound moves through that compartment. Thus, the model can be used to estimate not so easily measured values, such as AUC of the liver blood concentration. This may allow the investigators to improve their selection of an appropriate effective dose because the effective dose can be estimated for a wide range of doses.

Physiologically based pharmacokinetic models also provide a rational basis for the extrapolation of disposition data across species because they are based on actual anatomical and physiological values and on the fact that mammalian species are inherently similar. By scaling biochemical parameters and substituting anatomical and physiological parameters, PBPK models validated for one species have been successfully used to predict disposition patterns in other species and for humans (23). This is especially important in the area of risk assessment when one is trying to estimate what the true human disposition pattern will be.

Uncertainty in Physiologically Based Pharmacokinetic Models

The parameter values used in PBPK models are averages. The variability of parameter values is dependent upon overall species variability. The sensitivity of model predictions to changes in parameter values must be evaluated, for each parameter and each predicted value, so that model robustness can be determined. Quantitative as well as qualitative methods to systematically adjust for uncertainties in PBPK model parameters are needed.

Monte Carlo simulation methods represent one particular statistical approach to estimating overall effective dose variability due to uncertainties in model parameters (R. H. Reitz, C. J. Portier, personal communication). Substitution of average parameter values with their upper and lower confidence interval values, followed by recalculation of the model, is one method of determining model sensitivity. The problem arises in the choice of appropriate confidence intervals. Large intervals, chosen to account for extreme individuals, may dramatically change model predictions, and the resulting risk estimates, at the expense of accounting for relatively few individuals. A consistent, rational strategy must be developed to deal with differences in risk estimations, i.e. uncertainty in parameter estimates, based on the same PBPK model and data. Precise quantification of such statistical adjustments can be difficult, but they are ultimately necessary to provide proper incentives for PK data generation and its efficient utilization by regulators.

Lead

There are few extant examples of the full use of pharmacokinetic information in the initial safety assessment of food or color additives or food contaminants, DCM being one of the few exceptions. But there are examples of regulated substances of concern (e.g. lead, cyclamate, dioxin) where we have

come to appreciate that the traditional approach which ignores such information can be misleading, inadequate, and expensive.

In 1974, an FDA survey of heavy metals in food found relatively high lead concentrations in metal-canned foods (24). The lead-soldered seam of 'tin' cans was identified as the major source of this additional lead in canned food—particularly evaporated milk—and the FDA took steps to reduce this contamination (25). However, the new levels of acceptable exposure were based on data derived from animal studies and from adult humans. But we now know that in the adult human the bioavailability of lead in the typical diet is 10–15% and the bioavailability in infants and children is close to 50%, indicating a higher exposure rate in infants and children for a given food intake (26). Although the daily adult intake of lead through foods has been considerably reduced, from 100–500 μg/day during the 1940s to from 50–150 μg/day today, current levels are still uncomfortably high for many infants and children (27, 28). It has been reported that, in children, a lead blood concentration as low as 1.0–1.5 μg/ml " . . . is associated with the onset of effects that may be argued as becoming biomedically adverse . . ."[29]. These blood concentrations are achievable in infants at lead intake levels well within the 50–150 μg/day range (30).

An additional complication in determining acceptable exposure levels of lead is that lead is readily scavenged from the bloodstream and deposited in sites, where it may remain stored or from which it may be reabsorbed into the bloodstream. Lead is deposited in both soft tissues and in mineralizing systems, such as teeth and bone. Bone accumulates lead at a significant rate for much of the human life span and may act as a depot from which sizable amounts may be mobilized during periods of physiological stress, such as pregnancy, lactation, or chronic disease. The interplay between ongoing external exposure and exposure due to resorption from bone back to blood needs to be considered in assessing safe levels of exposure. These factors, as well as new information on the greater sensitivity of young children and the fetus to lead toxicity, are forcing health protection agencies to reassess their concerns regarding the threat to health posed by lead exposure (29). The FDA has recently proposed regulating the use of lead-glazed ceramic food-service pitchers and decorative ceramic ware for food use in order to reduce lead exposure from foods—a source of exposure once considered of lesser concern (31).

Cyclamate

Cyclamate is a nonnutritive sweetener already widely used in Canada and many European countries and currently under review by the FDA for use in the United States (32). Several of the scientific issues are pharmacokinetic in nature and PK data are vital in order to have confidence in establishing an

ADI. The bioavailability of cyclamate following oral administration is very low in humans and several species of animals. The fraction of cyclamate absorbed is rapidly excreted unchanged and there is little accumulation in organs, milk, and blood. However, a small but highly variable fraction of the unabsorbed cyclamate is converted to cyclohexylamine by a variety of microbial organisms dwelling in the colon and cecum (33). The cyclohexylamine thus formed is rapidly absorbed and is believed to be the toxic agent which produces testicular atrophy when cyclamate is ingested by rats (34, 35). These circumstances generate three distinct pharmacokinetic questions: (*a*) Is the rat a poor choice of test animal because of differences in its distribution and metabolism of cyclohexylamine? (*b*) What is the total amount of conversion of cyclamate to cyclohexylamine in humans? (*c*) What is the rate of conversion of cyclamate to cyclohexylamine in humans?

The first question is important to the sponsor because the rat appears to be anomalously sensitive to cyclohexylamine, and therefore the ADI based on rat data may be an underestimate. Mice did not exhibit testicular atrophy when tested at the highest dose tested in rats. Also, data indicate nonlinear and slower clearance in rats (as compared to mice) and correspondingly excessive accumulation of cyclohexylamine in both the plasma and testes in rats (34, 35). An ADI based on the mouse or on appropriately compared plasma concentrations would permit greater use of the substance if approved. The answer to the second and third questions is vital to assure the safe use of cyclamate in a large, variable, human population. The conversion of cyclamate to cyclohexylamine is unusually variable as it is dependent on the state of the intestinal flora which, in turn, depends on diet, the content of the colon, and the extent of microfloral adaptation to these conditions. The rate and total amount of cyclohexylamine is of regulatory concern not only because cyclohexylamine may cause testicular atrophy, but because the oral administration of cyclohexylamine was also found to have a hypertensive effect in humans (36, 37); determination of whether or not sufficient amounts of cyclohexylamine would be produced in humans to cause this effect is important. The FDA has encouraged the submission of this pharmacokinetic information. We agree with Roberts & Renwick who conclude: "Comparison of the circulating plasma concentrations of cyclohexylamine in man with the plasma and testicular concentrations in rats fed cyclohexylamine diets would avoid most of the unknown pharmacokinetic variables inherent in interspecies comparisons and provide[s] a more secure understanding of the safety factor" (35).

Dioxin

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are toxic contaminants found in trace quantities virtual-

ly everywhere in the natural environment. The average human daily intake of 'dioxins' is approximately 1–2 pg/kg/day in most countries and comes principally from food, e.g. milk, fish, meat, and other fatty foods (38). These accumulate in the body resulting in normal body levels in the 60–80 nanogram range. Of the hundreds of congeners, only 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been toxicologically tested in any detail and it is extraordinarily potent in animal studies, causing cancer, reproductive damage, and immunological effects at levels in the nanogram per kilogram range (39). TCDD's potential effects in humans are still controversial; only the occurrence of chloracne is well established (40).

One of the major difficulties in assessing human health risks is the inherent inaccuracy in exposure estimates. Recent efforts to develop a quantitative biological marker of TCDD intake by using its long half-life in humans have met with success and offer an accurate means of estimating exposure, even many years after the event. Initial demonstration of TCDD's long half-life in humans, 2,120 days, was unexpected in view of the relatively rapid elimination in rodents (2–4 weeks) (41). But more recent studies have confirmed that the half-life for TCDD in man is approximately 7–7.5 years (42, 43). Plasma lipid concentrations were found to be representative of adipose concentrations, a major storage site of non-volatile halogenated compounds; the partition coefficient of TCDD between plasma and adipose tissue on a lipid basis is approximately one (42). Thus it is possible to estimate the extent of the original exposure by extrapolating backward from current dioxin plasma levels.

Whether in the design, conduct, or interpretation of safety studies, it is evident that pharmacokinetic concepts can play a vital role. Certainly concepts such as absorption, distribution, and elimination have always been basic to toxicological judgment. However, to treat pharmacokinetics as simply a more systematic or quantitative approach to traditional toxicological or physiological ideas is too vague a definition of its potential use in toxicological testing. Clearly, the quantitative measurement of pharmacokinetic parameters, from those as easily determined as AUC to those more theoretically based such as V_{max} and K_m, are not routinely submitted by firms requesting food additive approvals nor are they demanded by the FDA. We have tried to indicate why this is so—primarily because of the traditional reliance on animal studies alone and the use of large safety factors. But the situation is changing and it seems to us that the role of pharmacokinetics will increase. There are several reasons for this:

1. The increasing role of cancer-risk assessment and regulations acknowledging and permitting some level of risk. This places a demand on greater quantitation of that risk and emphasizes the need for better measurement of effective dose.

2. The increasing need to understand more about a chemical's mechanism of action prior to major corporate commitment. The increasing costs associated with mistakes regarding a chemical's prospects in the regulatory arena are demanding a better and deeper understanding of possible toxic effects.
3. The desire to expand the use of a successful additive beyond the limits provided by the original approval and the ADI based on the less refined toxicology studies.
4. The advent of novel foods for which conventional toxicological methods are inappropriate, e.g. noncaloric fats that comprise a large portion of the daily diet. These will require human clinical investigations that can be better designed using preliminary animal studies and pharmacokinetic principles.

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