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PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELS IN RISK AND EXPOSURE ASSESSMENT

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Summary. - *Pharmacokinetic non-linearities occur between different doses and between different species. Physiologically based pharmacokinetic models are accurate tools for taking these non-linearities into account. Dichloromethane and perchlorethylene are two examples discussed in this paper. For dichloromethane the pharmacokinetic non-linearity factor results in a greater delivered dose than would be predicted from linear relationships as the dose increases. For perchloroethylene the opposite holds true. In addition a brief illustration of the use of pharmacokinetic models as tools for interpreting biomarker data is provided.*

KEY WORDS: physiologically based pharmacokinetic models, dichloromethane, perchloroethylene, biomarkers of exposure.

Riassunto (Modelli farmacocinetici a base fisiologica per la stima del rischio e dell'esposizione). - *Non-linearità di tipo farmacocinetico possono presentarsi tra dosi differenti e specie biologiche differenti. I modelli farmacocinetici a base fisiologica sono degli strumenti accurati per la considerazione di queste non-linearità. In questo lavoro sono discussi i due esempi del diclorometano e del percloroetilene. Nel caso del diclorometano, la dose somministrata risulta maggiore di quella stima sulla base di una relazione lineare in rapporto all'incremento della dose: questo è il fattore di non-linearità. Per il percloroetilene accade l'opposto. E' anche fornita una breve illustrazione dell'uso dei modelli farmacocinetici quali strumenti per l'intrerpretazione dei dati dei biomarker.*

PAROLE CHIAVE: modelli farmacocinetici a base fisiologica, diclorometano, percloetilene, biomarker dell'esposizione.

Introduction

Pharmacokinetics is the study and description of the processing of chemical compounds by living organisms. The movement of compounds throughout the body is a kinetic rather than static process. Foreign molecules and endogenous biomolecules exhibit, like all other molecules in the universe, continuous motion. Thus, the representation of the interaction of xenobiotics and biologic tissues as a static process is an inaccurate over simplification. Within the eye of our imagination it is easy to "see" foreign invaders cross membranes, floating in the blood, attaching themselves as free riding passengers onto the surfaces of cells, organelles, and receptors. They may readily embrace and interact with the very enzymes and molecules that maintain life itself.

Pharmacokinetics is description of the time course disposition of a xenobiotic, its biotransformed products, and its interactive products within the body. It includes a description of the compounds's absorption across the portals of entry, transport and distribution throughout the body, biotransformation by metabolic processes, interaction with biomolecules, and eventual elimination from the body.

Pharmacokinetic (PK) analyses or assessments can be used in two very general ways. First, they can be applied for forward analysis. Such PK analyses use exposure data to calculate biologically meaningful measures of dose. Second, they can be applied for reconstructive dose assessment. In this case data on measured biomarkers or tissue concentrations of a compound and/or its metabolites are used to calculate total dose of a xenobiotic received by an organism.

There are several ways to perform such analyses but physiologically based pharmacokinetic (PBPK) models are the emerging technique of choice. These models include a series of mass balance equations which describe the total disposition of the xenobiotic within the body. These models can be formulated as complexly or simply as the data allow or necessitate. For example, models have been written [1] that describe the incorporation of products of xenobiotic metabolism within the sub-cellular organelles of brain cells. Typically, much simpler models are implemented. Regardless of the level of complexity,

the fundamental characteristic of PBPK models is their dependence upon parameters with physiologic meaning and their description of actual anatomic compartments. In contrast, the more traditionally used classical models, while also useful, depend on rate constants describing the transfer of xenobiotics between a few arbitrary compartments. Although simple mathematical manipulation shows the two types of models to be meaningfully related, the utility of the classical models tends to be for descriptive rather than predictive purposes. In particular, they do not lend themselves easily for interspecies extrapolations of dose. Physiologic models, because of their more direct and accurate description of the actual anatomy and physiology, lend themselves to being better predictors of target level dose within and between species.

Modeling and interpretation

Fig. 1 shows a diagramatic representation of one such PBPK model. It should be remembered that the parameters regulating transport into each compartment are actual physiologic parameters. The arterial blood flow to each organ and real organ volumes make up important parameters in this particular model. Under different circumstances these parameters would be replaced, as necessary, by others such as permeability coefficients. Of course, any metabolism would also be described in detail. Such physiologic and biochemical parameters are determined from knowledge gained in experiments performed as needed. There is a wide body of reference values for many parameters such as organ sizes and blood flows, for example. The determination of metabolic rate constants may require case specific experimentation.

In the simplest risk assessment a dose-response function is calculated using the applied dose. Human exposure levels are then compared to the animal dose and some prediction of risk to the human is estimated from the previously calculated animal dose-response function (Fig. 2).

A slightly more complicated and perhaps realistic approach (depicted in Fig. 3) takes into account the absorbed dose by incorporating absorption fraction in the calculation. In this case the animal dose used for the dose-response function is the absorbed dose rather than just the applied dose. For example, in Fig. 4 it can be seen how ventilation rate, absorption fraction, and duration of exposure can be used to determine the dose actually entering

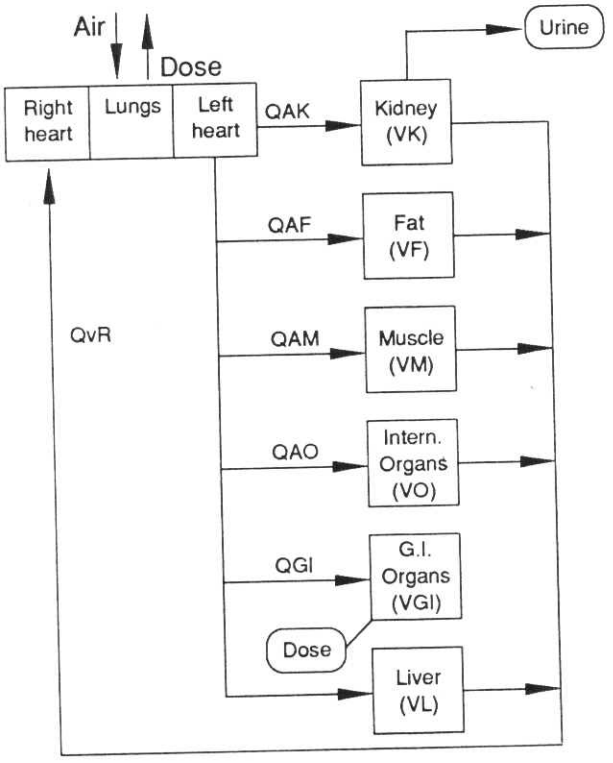

Fig. 1. - Schematic of a sample physiologically based pharmacokinetic model. QA: arterial blood flow to an organ. V: volume of an organ. K: kidney. F: fat. M: muscle. O: various internal organs "lumped together". GI: gastro-intestinal organs. L: liver. QvR: flow of venous return.

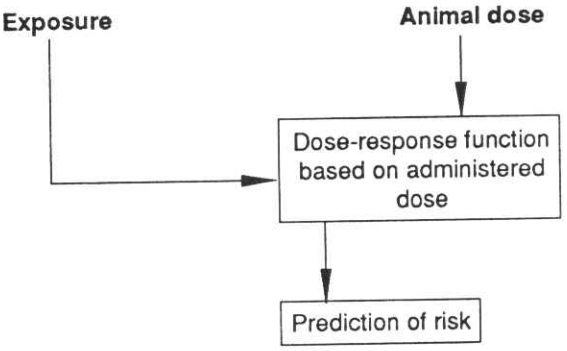

Fig. 2. - Risk prediction based on only animal dose and human exposure data.

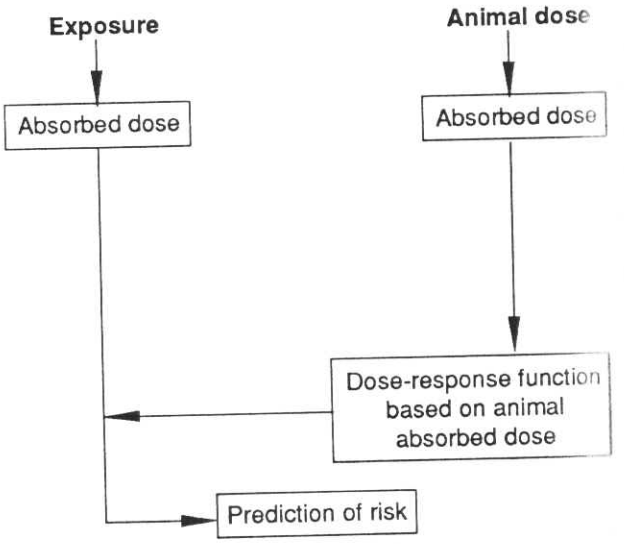

Fig. 3. - Risk prediction based on animal and human absorbed dose

REVIEW EXAMPLE TO ILLUSTRATE
GOAL OF PK ANALYSIS

Concentration in air:	0.686 mg/CuM
Ventilation rate:	20 CuM/day
Duration of exposure:	5 hours
Absorption fraction:	0.6

20 CuM/day x 1 day/24 h x 5 h x 0.6 x 0.686 mg/CuM =

ABSORBED DOSE: 1.175 mg

IDEA: ABSORBED DOSE MAY BE A BETTER MEASURE FOR HEALTH EFFECTS THAN EITHER CONCENTRATION x TIME OR INHALED DOSE

Fig. 4. - Example of use of minimal pharmacokinetic (PK) data. CuM: cubic meter. h: hours. mg: milligram.

the body. When data regarding absorption fraction are lacking it has been assumed that the absorption fraction in the exposed animal is equal to that in the test animal.

Absorbed dose may be thus calculated for both the test species and the species at potential risk. The absorbed dose is then used in dose-response functions for calculating predicted risks. Still, the fundamental problem with this approach is that none of the inter-route, inter-dose, and inter-species pharmacokinetic non-linearities are taken into account. Further, the surrogate for the critical dose is taken to be the total absorbed amount by the body with no consideration for how much is delivered, or in cases of biotransformation, formed and delivered, to the actual target sites within the body. In sum then, non-linear processes are not accurately taken into account and target tissue doses are not estimated and then not used as a surrogate for critical dose. Thus considerable uncertainty still resides with the risk calculated from these "doses".

Fig. 5 illustrates the concept of using greater and more detailed knowledge of pharmacokinetics to improve the dose assessment component of the risk assessment process. In this case the actual delivered dose to specific organs and cells is calculated. The total pharmacokinetic process, including biotransformation for activation and deactivation is considered and quantitatively taken into account. PBPK models, developed from extensive data, are often the method of choice for this purpose. This process can demonstrate inter-species non-linearities in the pharmacokinetics. Likewise, with this thorough understanding, the PK differences between doses and exposure routes can be explicitly demonstrated and quantified.

Although a thorough understanding of the PK and the resultant PBPK model greatly improves the risk assessment process by more accurately estimating dose, several uncertainties and limitations remain. For example, often multiple measures of internal dose, such as parent compound, metabolites, adducted compound, or receptor bound product, are available. Without considerable knowledge regarding the mechanism of action it is not clear what measure to call the effective dose. Also, the estimation and interspecies comparison of metabolic rate constants is difficult and sometimes relies heavily on untested assumptions.

Even with such knowledge on the mechanism of action and an understanding of the governing processes of the particular metabolism involved, pharmacodynamic differences between species are not easily understood. That is, it is yet to be clarified if pharmacokinetically equivalent doses result in equivalent risk between different species, or for that matter, between different individuals of the same species. As a result, various empirical and sometimes arbitrary scaling factors are used and must be compared. This uncertainty with pharmacodynamics is the next area of research on the horizon which must be vigorously pursued.

Such analyses have been applied in an effort to get better delivered dose estimates. Two slightly different applications can be illustrated using the chlorinated hydrocarbons dichloromethane (DCM) and tetrachloroethylene or perchloroethylene (PERC). Both chemicals cause tumors in animals and both are believed to depend on metabolic biotransformation to be tumorigenic.

The evidence for dichloromethane shows that it induces mouse lung and liver tumors and benign mammary gland and malignant salivary gland tumors in rats. It

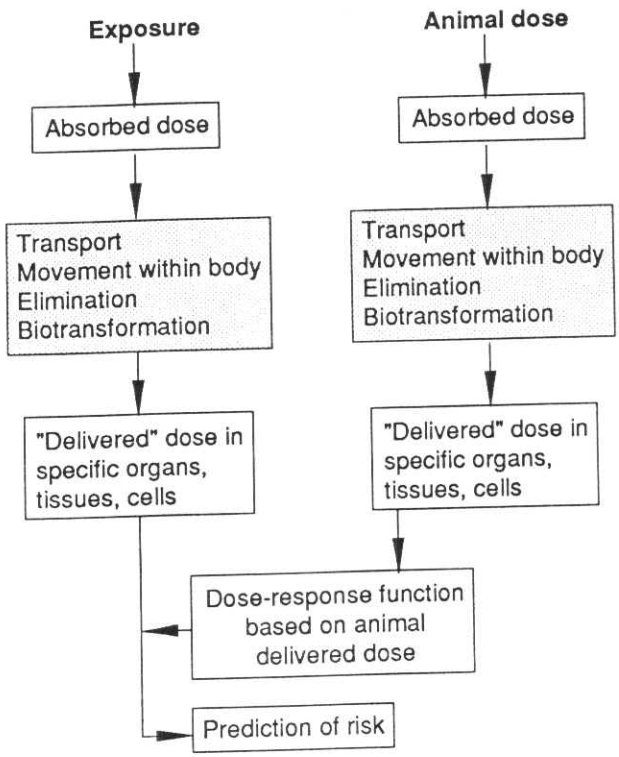

Fig. 5. - Risk prediction based on animal and human pharmacokinetic (PK) information.

appeared to not induce tumors in hamsters. Two metabolic pathways are operational. One, mediated by the mixed function oxidase (MFO) system produces carbon monoxide and carbon dioxide. The second, a glutathione-S-transferase (GST) mediated path produces carbon dioxide and no carbon monoxide. The GST mediated path appears to be primarily responsible for the lung and liver tumors through a reactive intermediate. Although some tumorigenic potential of the intermediates produced by the MFO pathway cannot be ruled out the weight of evidence suggests a far more dominant role for the GST mediated pathway. Thus the amount of metabolite produced by this pathway was used as the surrogate for effective dose. The MFO pathway was shown to saturate at typical exposure levels while the GST mediated pathway was considered to be linear at the same exposure levels. It showed, however, much less affinity for the substrate. Hence toxic activation by the GST mediated path appeared quantitatively most significant at higher doses. There may be some inaccuracies in this model, as more recent data may reveal, but the purpose here is an illustrative one only.

At the time the assessment was performed for PERC, considerably less was known about the details of its mechanism of action and metabolism. It was fairly well documented that metabolic biotransformation was also necessary for tumorigenesis in this case. Although other tumor sites were demonstrated, liver tumors were considered as the endpoint. As such, total metabolite produced, predominantly by P-450 oxidation, was used as the surrogate for effective dose.

The actual details of both models and their development, and resultant impact upon unit risk estimates are presented elsewhere [2-5]. Here some illustrations of their practical uses are presented.

Fig. 6 shows the impact of pharmacokinetic non-linearities as exposure concentration increases. A PK factor of 1 would indicate that the surrogate for effective dose at 500 ppm exposure concentration is simply 5 times the amount at 100 ppm. Inspection of Fig. 6 quickly reveals that while neither chemical exhibits linear PK their respective deviations are quite different from one another. In the case of DCM the amount of surrogate formed with increasing dose is greater than would be predicted by a linear assumption. For PERC on the other hand the amount of surrogate formed is somewhat less with increasing dose than would be expected from the simple linear assumption.

Fig. 7 illustrates this impact upon risk. This is a plot of exposure concentration *versus* arbitrary risk. The arbitrary risk is taken as the risk determined at some low exposure concentration (i.e. the "unit" risk) and multiplied by exposure concentration and the PK factor. The PK factor is equal to 1 for the linear assumption. The PK factors for the DCM and PERC cases were calculated from PBPK model output. It can easily be observed that, based on the particular model output used here, the linear assumption would under estimate the DCM risk by 2.4 fold (11.6 risk at 500 ppm for the DCM modeled case divided by 4.8 risk at 500 ppm for the linear case). Similarly the linear assumption would over estimate the PERC risk by 0.64 fold.

Next I will illustrate the use of PBPK models to compare the possible risk resulting from different exposure scenarios. Again, for demonstration purposes only the PBPK model developed from human exposure to PERC is used. Fig. 8 compares exposures of 400 ppm for 2 h *versus* 800 ppm for 1 h, *versus* 100 ppm for 8 h. This exercise asks the question if equivalent time X exposure concentration products (800 ppm-h) result in equivalent internal doses. Fig. 8 shows the liver concentration of parent compound resulting from the three different exposure regimens.

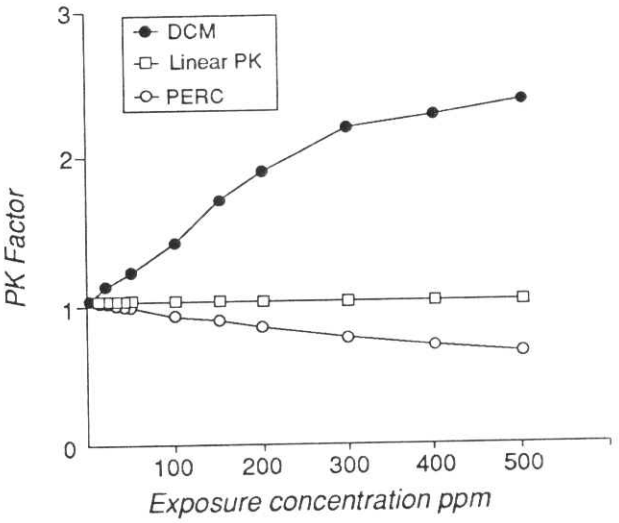

Fig. 6. - PK non-linearity factors. (For PERC and DCM as predicted by PBPK models). PK factor: the degree of non-linearity with increasing exposure concentration as calculated by the PK model.

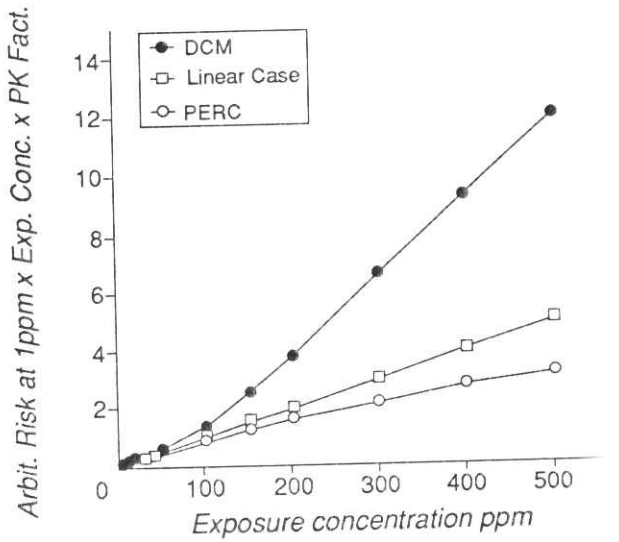

Fig. 7. - Effect of the PK non-linearity. (On the delivered dose of PERC and DCM as predicted by PBPK models).

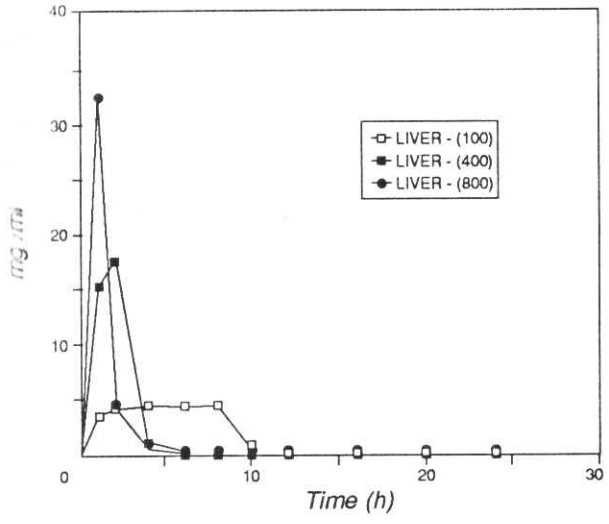

Fig. 8. - PK model predictions for liver concentrations at three exposure regimens.

Differences in both peak levels and profiles are observed. Peak heights are proportional to exposure concentrations for these three scenarios. The peak is maintained for the exposure duration, hence the observed difference in profiles.

Fig. 9 provides a similar comparison for the adipose tissue. For this tissue the differences are less striking. The actual peak levels reached are virtually the same for all three cases as are the elimination profiles after the expsoure period has ceased. Thus, if the concentration in this tissue were to be used as a surrogate for effective dose all three scenarios are nearly identical.

Fig. 10 shows the comparison for the area under the concentration curve (AUC) of PERC in the liver. In this case it can be observed that the 800 ppm/1 h exposure regimen delivers the greatest amount of surrogate. Fig. 11, on the other hand, shows a similar comparison for the totatl amount of metabolite formed. In this case the 100 ppm/8 h exposure delivers the greatest amount of surrogate. Remember from Fig. 8 that the level of PERC in the liver was lowest for that exposure scenario. This level is below the saturation point of the bioconverting enzymes, thus a larger percentage of the total PERC present is converted than for the other two scenarios. Given this and the longer exposure period a greater conversion to metabolite is realized.

This next exercise has been formulated solely for illustrative purposes to show how PK data and models can be used in conjunction with biomarker information to help assess exposure. As such, the biomarker and dose data

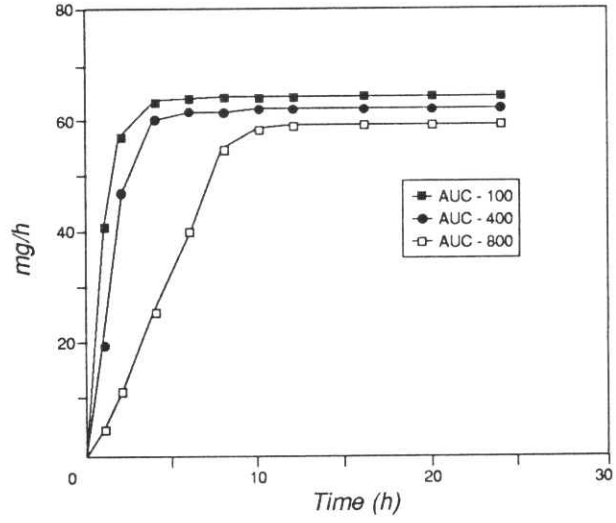

Fig. 10. - PK model predictions for area under the concentration curve at three exposure regimens.

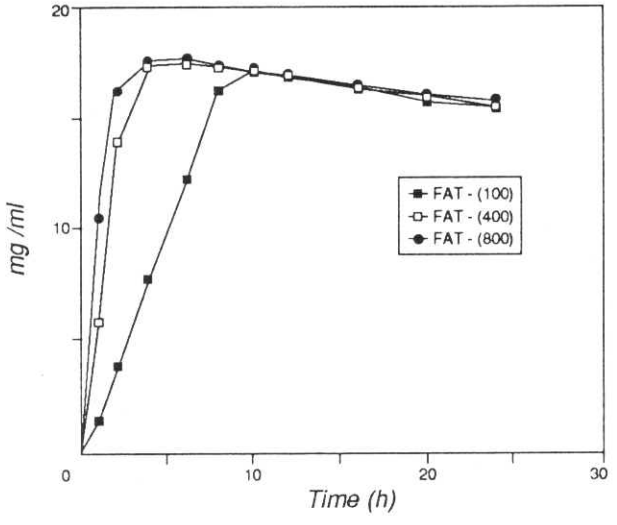

Fig. 9. - PK model predictions for fat concentrations at three exposure regimens.

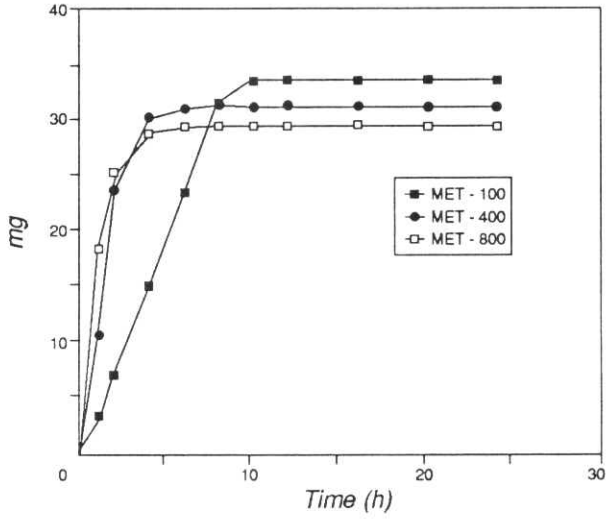

Fig. 11. - PK model predictions for the amount of metabolite formed at three exposure regimens.

were essentially manufactured from past experiences and knowledge. Under actual exposure conditions the formulation and validation of the PBPK model could be quite involved and complex. Usually such models are quite data intense. If models and appropriate data are lacking they must be generated simultaneously with, or even in anticipation of, the biomarker development. As will be illustrated, more than one biomarker and associated analysis tools may be necessary to get accurate estimates for large exposure ranges. If estimates of the exposure ranges are known *a priori* it may be possible to reduce the number of necessary biomarkers required.

Discussions of actual model formulation procedures will vary according to the specific case and are far beyond the scope of this paper. The same can be said for the mathematical procedures for solving the mass balance equations of the PBPK model. Suffice it to say that, to date, complicated models are solved numerically rather than analytically. The expanding capacity and speed of computers has enabled the solution of models by what were previously considered to be tedious numerical methods to become common place.

Model simulated output is then used with biomonitoring data from subjects to estimate dose. Again, a variety of methods can be used. The most complicated, but perhaps the method affording the most accuracy and resolution between doses, uses the PBPK model itself. Biomonitoring data are taken and the model is then run against these data. Numerical techniques are thus used to estimate the dose that could have resulted in the monitored biomarker data. Given proper numerical methods and appropriate software, confidence values around the estimated dose can be calculated. In this manner the more reliable and accurate monitoring data will result in dose estimates that are associated with greater confidence.

A contrasting and less computationally intense method involves using the PBPK model to give outputs at several arbitrary dose ranges. The model outputs are then graphed and the resultant graphs can be used as reference graphs from which to estimate dose ranges after biomarker monitoring is performed.

A third approach is a compromise between the first two. For this case model outputs at several dose ranges are put into a computer data bank. An expert system, with access to that data bank, is then employed to compare the monitored data to model simulated outputs and thus give estimates of dose. Such a computer based expert system could be formulated to perform appropriate statistical analyses of multiple data points of the monitored marker.

Each is advantageous under specific conditions. Following is a simple illustration of the second, or graphical approach.

Figures 12 through 14 illustrate the use of pharmacokinetic information to determine dose in one particular exposure scenario. Eight possible dose levels (1-8) are included in this illustration. Two things are assumed (with *a priori* knowledge) about the scenario. First it is assumed or known that exposure is still ongoing and second that the plateau or steady state has been reached.

Three different biomarkers have been monitored. Fig. 12 shows the profile of marker 1 for the 8 dose levels. The left panel shows the marker's concentration with time profile as determined from the pharmacokinetic analyses for the various doses. The panel on the right shows the steady-state concentrations (Css) with increasing dose levels (1-8). If the monitored marker level is less than 5.0 a dose range can be established. For example, from the left panel it can be observed that if the concentration level (y-axis) of marker 1 is 4.9, then the dose must then be between level 2 and level 3 (as dose 2 has a biomarker concentration of 3.0 and dose 3 has a biomarker concentration of 5.0). The resolution could then be increased by increasing the number of simulated model outputs within that range.

If the marker level were greater than 5 it would not be possible to discern between dose levels 3 through 8, as due to some saturating effects all of these higher dose levels should correspond to the same concentration of this biomarker. Fig. 13 is the analogous graph for marker 2. Inspection reveals that this marker can discern between the first four dose levels rather than just the first three.

One might wonder why to use marker 1 at all when marker 2 is adequate for a wider dose range. It may be that marker 1 is more accurate at lower doses or that it is easier and cheaper to obtain and analyze. Thus it may be the preferred marker for screening or for determining the lower dose levels.

Fig. 14 repeats the process but for marker 3 instead. Inspection here revealed that this marker can discern between dose levels 5 through 8. It also demonstrates that this marker, due to its kinetic profile, is incapable of being used to estimate the lower dose levels.

Conclusions

These illustrations teach us several things. First, we must remember that risk assessment is part art, part science and engineering, and part intuition. Thus, we must look to use all of the available information with all of the available tools for analysis of that information. We learned from many of these cases, particularly the DCM case, that the whole is indeed greater than the sum of its parts. No one piece of information alone can shed light on the puzzle, but taken together much can be concluded about potential risk. For example, the exact mechanism of DCM's tumorigenesis remains unelucidated; however, taken with the pharmacokinetic information, at least the pathway primarily responsible was identified. Still uncertainty remains. Could the other metabolic path contribute to this tumorigenicity in some quantitatively small way, so that our conclusion cannot be totally verified? The solution to this quandary lies in remembering that all of our previous risk assessments contained considerable uncertainty. The

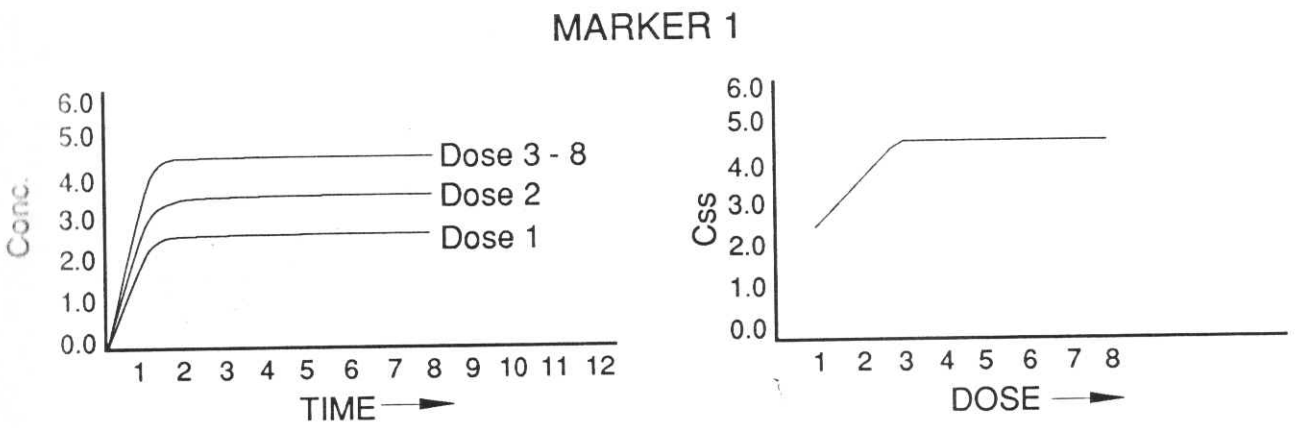

Fig. 12. - Hypothetical biomarker concentration after exposure to a chemical at 8 possible exposure levels.

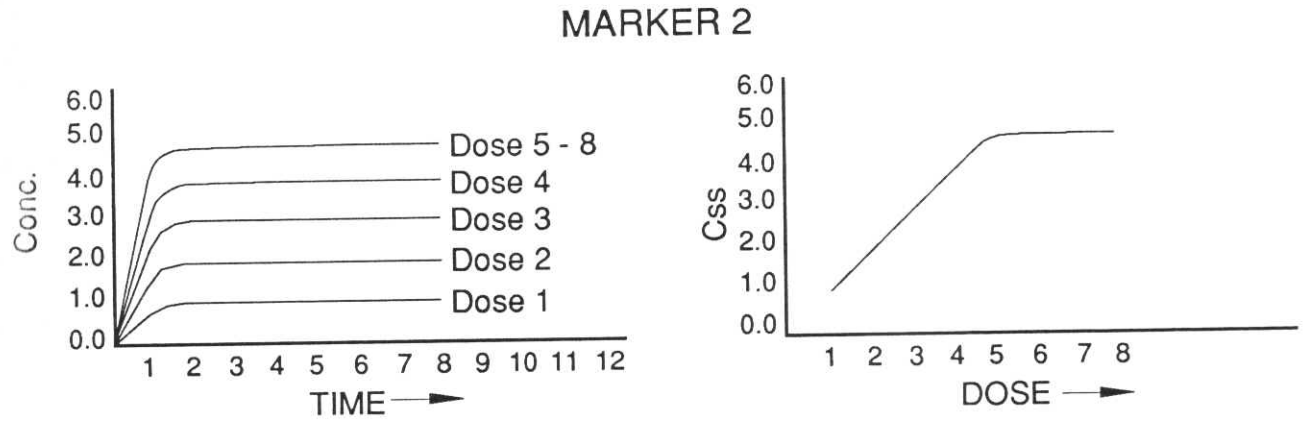

Fig. 13. - Hypothetical biomarker concentration of a 2nd marker after exposure to the same chemical as in Fig. 12 at 8 possible exposure levels.

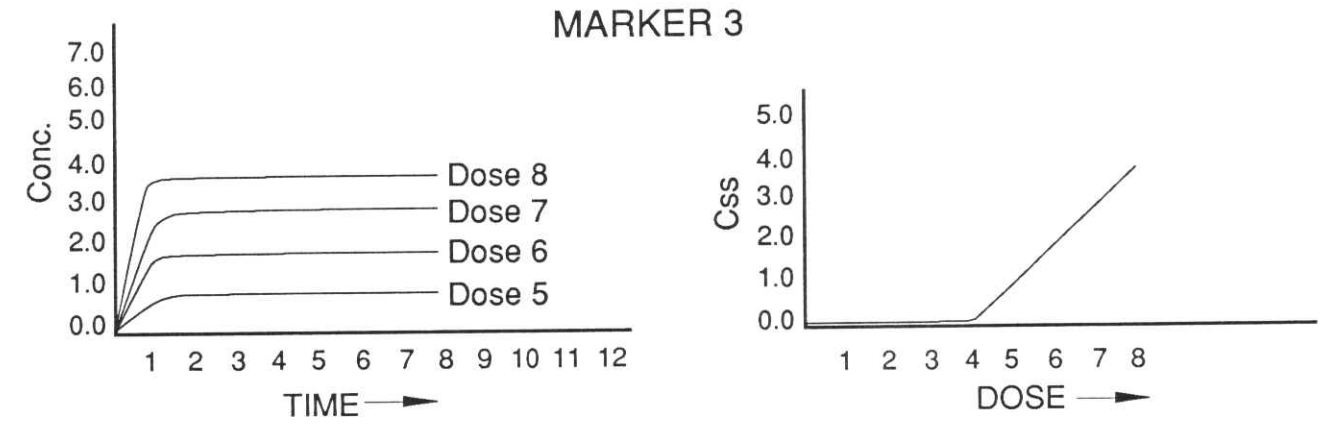

Fig. 14. - Hypothetical biomarker concentration of a 3rd marker after exposure to the same chemical as in Fig. 12 at 8 possible exposure levels.

difference here is that with biologically based procedures the source of the uncertainty can be more easily identified and quantified. Statisticians were able to tell us for example, what the highest possible contribution that could be made to the tumorigenesis process by the MFO pathway, given the data and the pharmacokinetic information. From that estimate and the PK information, a value for the upper bound risk containing that possibilty could be made. One no longer needs to blindly assume that this MFO pathway could possibly contribute as much as 100%. In fact even with simple illustrations, as provided by the model based dose assessment for DCM, a reasonable level of contribu-

tion could be assigned to a "shadow" pathway consistent with available, toxicological and pharmacokinetic evidence.

Another lesson learned by these real world examples is that none of us can afford to work in isolation and in secret from one another. The DCM case, in particular, was an example of a large cooperative effort between experimentors, modelers, and risk assessors. In addition, the effort was accomplished by continous interaction between industry and government scientists. As data were gathered they were discussed by groups from both industry and regulatory agencies. Interpretations were exchanged and further necessary experiments were identified and then performed. The process continued over more than two years and the more rational biologically based dose assessments were the result. I would venture to say that without such a concerted effort on the part of Imperial Chemical Industries, the Dow Chemical Company, the US Consumer Product Safety Commission, the US Food and Drug Administration, and the US Environmental Protection Agency the process would not have occured. The universe of technical knowledge expands too rapidly and the costs of experimentation and analysis increase too greatly for any of us to work in a vacuum. Time is precious. Much valuable time would be consumed before one person or even group could come to rational answers while working alone.

Likewise, we must quickly realize that different disciplines each can contribute valuable parts to the whole process. Again, the whole is greater than the sum of its parts. When molecular biologists see the basic cellular and sub-cellular process they see adduct formation, receptor binding, and oncogene activation. Kinetists see reaction rates, Minot models, allosteric binding sites altering kinetic rates, Michaelis-Menten and higher order kinetics describing fundamental reactions. Are we looking at different things or are we looking at the same things differently? I suggest the latter and further I suggest that together we must look at the same things from all the different perspectives. Let us not be afraid to reach out to the new. Yes, new techniques frequently bring on new questions. I expect that the Hubble telescope now orbiting our globe will answer some of the queries that astronomers have pondered for years but it will also raise new and more perplexing questions. But our hope for it and for all of our scientific queries is that in addition to answering some questions and raising others these queries identify and help us measure the uncertainty. Remember that the old way of doing things sometimes seems more certain only because we lack the knowledge to see what we truly do not understand. Let us not either curse the darkness nor accept it, but rather, let us light the candle and move it forward as we travel.

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