Use of Anatomical and Kinetic Models in the Evaluation of Human Food Additive Safety

Submitted: September 23, 2004; Accepted: September 27, 2004; Published: September 22, 2005

William L. Roth¹

¹US Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD

ABSTRACT

Toxicological testing in animals is relied upon as a surrogate for clinical testing of most food additives. Both animal and human clinical test results are generally available for direct additives when high levels of exposure are expected. Limited animal studies or in vitro test results may be the only sources of toxicological data available when low levels of exposure ($\mu\text{g}/\text{person}/\text{day}$) are expected and where no effects of the additive on the food itself are desired. Safety assessment of such materials for humans requires mathematical extrapolation from any effects observed in test animals to arrive at acceptable daily intakes (ADIs) for humans. Models of anatomy may be used to estimate tissue and organ weights where that information is missing and necessary for evaluation of a data set. The effect of growth on target tissue exposure during critical phases of organ development can be more accurately assessed when models of growth and known physiological changes are combined with pharmacokinetic results for test species. Kinetic models, when combined with limited chemical property, kinetic, and distribution data, can often be used to predict steady-state plasma and tissue levels of a test material over the range of doses employed in chronic studies to aid in interpretation of effects that are often nonlinear with respect to delivered dose. A better understanding of the reasons for nonlinearity of effects in animals improves our confidence in extrapolation to humans.

KEYWORDS: species extrapolation, growth models, toxicokinetics, food additive safety

INTRODUCTION

The Food, Drug and Cosmetic Act of 1958 and amendments require the Food and Drug Administration (FDA) to establish the safety of “all substances ... the intended use of which results or may reasonably be expected to result ... in their becoming a component of food or otherwise affecting the

characteristics of food.”¹ The wide range of substances to which this definition applies (Table 1), including unintentional indirect additives, generally precludes clinical testing of most chemicals defined as food additives. The extent of safety testing in animals is limited from an economic standpoint as well, since the quantities of many indirect additives produced is small in comparison with those intentionally added to food (direct additives). As a consequence of testing limitations, mathematical extrapolation from results obtained in animals is a key step in the prediction of safety in humans.

Estimation of a level of exposure that is safe invariably translates to finding an acceptable mathematical description of the dose-response curve. In cases where exposure to a food additive is low, and/or limited data are available, safety determinations are based on very conservative assumptions and simple models (eg, benchmark dose, linear multistage model) that employ doses to animals in chronic studies in combination with safety factors^{3,4} to estimate acceptable levels of exposure to human consumers. The safety factors are intended to more than compensate for any differences in response between species, any nonlinearity in the dose-response curve, and any variance in sensitivity within the human population.

When the use of food additives with specific, desirable effects is proposed, exposure levels are generally higher. Establishing the safety of substances at higher levels of exposure requires a more extensive set of safety studies, including studies of the pharmacokinetics and metabolism of the food additive. While this places a greater burden on the manufacturer to conduct experiments and on the FDA to review larger volumes of data, it also creates the need to develop detailed models of the absorption, biological fate, and any physiological effects of the food additive in human consumers. Dose-response curves can be defined with much greater accuracy if pharmacokinetic studies for the new food additive are designed to overlap the dose range(s) used in toxicity studies.⁵ However, it is often possible and may be necessary to simulate the disposition of a chemical at higher or lower doses when the chronic study doses used are significantly different from the doses used in pharmacokinetic experiments.

Comparison of the metabolic fate and pharmacokinetics of a food additive between several species of test animals is often the principal means that FDA uses to decide which

Corresponding Author: William L. Roth, US Food and Drug Administration, Office of Food Additive Safety, 5100 Paint Branch Pkwy, College Park, MD 20740. Tel: (301) 436-1238; Fax: (301) 436-2965; E-mail: wrth@cfsan.fda.gov

Table 1. Major Food Additive Categories*

Category	Examples
Food preservative	Natamycin (antimycotic) tert-butylhydroquinone (antioxidant)
Coatings	Coumarone-indene resin Oxidized polyethylene
Polymers and components	Polyamides 1,4-benzenedicarboxylic acid (monomer)
Special dietary additives	Nicotinamide-ascorbic acid complex Xylitol
Anticaking agents	Calcium silicate Sodium ferrocyanide
Flavoring agents	α -amylcinnamaldehyde Methylacetophenone
Gums and related substances	Carrageenan Xanthan gum
Multipurpose additives	Aspartame/neotame (sweeteners) Polysorbates (emulsifiers)

*Categories and examples taken from 21 CFR 172–177.²

animal species is most similar to humans, and whether or not an effect observed in a single species of test animals is relevant to humans. Pharmacokinetic analyses may be used primarily for qualitative decisions, such as choosing representative species, or with better data and results from some clinical studies, to make quantitative estimates of acceptable levels of human exposure.

It should be noted that we use mathematical models as learning tools that allow us to (1) convert individual data points from a study into functions that can predict outcomes at points that have not been measured within the range of available data, (2) integrate results from multiple studies into smooth functions, and (3) incorporate untested hypotheses with unknown parameters into models with known parameters to determine the feasibility of different assumptions and the sensitivity of the problem in question to different parameters. Therefore, we do not consider the inability of a model to fit a set of experimental results to be a failure. Such a result simply means that we did not incorporate enough detail or we did not understand an important component of the pharmacokinetic/pharmacodynamic system. We make changes and try again. Without the model we might never have known that such a knowledge gap existed.

The following examples show ways in which both qualitative and quantitative uses have been made of animal growth models and pharmacokinetic results to better define the systemic dose and evaluate effects of new food additives.

USE OF ANATOMIC/GROWTH MODELS

Accurate extrapolation from the results of animal experiments to outcomes in exposed human populations requires

accurate translation of anatomic, physiologic, and biochemical characteristics between test species and humans. Extrapolation of animal toxicity results to humans requires a showing that pharmacokinetic properties and systemic doses of a food additive are similar between test animals and humans. There is generally no one-to-one equivalence between species, and several types of computations must be conducted in order to place boundaries on extrapolations. The first computation we make in reviewing pharmacokinetic data is to determine the mass balance,⁵ which is the sum of the masses of tracer in all volumes of distribution and excretion. In some cases, a study is submitted in which tissue concentrations (C_i) of a tracer are reported, but the whole tissue/organ weights are not reported. The mass balance cannot be computed without tissue volumes (V_i) (mass = $C_i V_i$). If the age and/or body weights of the test animals are reported, a good estimate of the organ weight can be made from mathematical models of growth and body and organ weight.⁶⁻⁸

Anatomic/growth models are well developed for strains of rats commonly used in toxicological research. Less extensive data and models have been developed for mice and dogs (Figure 1). Models of this type can also be used to estimate the effect of feed restriction when reduced food intake in animals on chronic study complicates the interpretation of toxicity studies.⁷ When an adverse effect is observed in a specific tissue (target tissue), accurate computations of target tissue concentrations/doses often show dose-effect correlations that would otherwise be less distinct.

Luecke et al^{10,11} have used human embryonic growth models with doses and effects from animal teratology studies to predict periods of teratogenic sensitivity during human gestation. While the use of such models is limited by the availability of data sets useful for animal-to-human extrapolation, they provide anatomically accurate tissue volumes and physiological parameter values from which pharmacokinetic and pharmacodynamic results can be used to extrapolate effects across species.

PREDICTION OF SYSTEMIC DOSES FROM LIMITED KINETIC DATA

Time-to-Effect Versus Dose

It is well known that certain toxic effects can only be produced by long-term exposure to a chemical, either because the probability of the initiating event is low, or because a deficiency of some sort (eg, mineral or vitamin deficiency) must be produced before the physiological change underlying the effect can occur. Some examples of this type of situation are shown in Figure 2A. Laboratory rats and mice have been bred to achieve a variety of pathological characteristics, including spontaneous occurrence of cancer. Murray¹² described the incidence and time of appearance of mammary

Figure 1. Fitted body and organ growth curves from Roth et al⁵ and data of Deavers et al.⁹ Male beagle body weights were fitted to the Gompertz equation: $W(t) = W(0)\exp\{-(\beta/\alpha)[1 - e^{-t}]\}$. The parameter α is sensitive to food consumption and diet quality. The parameter β is constant for a species/strain. Fitted parameter values for male beagles were $\alpha = 0.0595/\text{day}$, $\beta = 0.0163/\text{day}$.

tumors in several strains of mice in an effort to separate genetic, hormonal, and viral effects on their appearance. For most healthy animals, including humans, cancer is a disease of old age, occurring after a lifetime of DNA damage, which results in the loss of replication control in some cells. Testing chemicals for cancer potency is limited by the tendency for many test species to develop spontaneous tumors late in life, as well as by the number of animals surviving long enough for tumor induction to be observed.

Druckrey et al¹⁴ demonstrated a linear relationship between the cumulative dose and duration of exposure (ie, Dose $\times$ t = Effect) for the appearance of liver tumors in animals treated with a series of nitrosamines, including diethylnitrosourea (DENU). At very high doses (~ 10 mg/kg-day) tumors appeared in 100% of the animals with only 4 months of treatment. When doses were reduced 50-fold (0.15 mg/kg-day), the percentage of animals with tumors did not decline; rather the time of appearance (induction time) increased to 20 months. Depending on the potency of the carcinogen, such tumors might not be induced within the animal's lifetime. Since the usual cancer bioassay period is 2 years,

Figure 2. (A) Mammary tumors do not appear in untreated C3H mice until 40% of the mice die from other causes (data from Murray¹²). Note that the mortality rate accelerates once tumors begin to form. (B) Expected Survival $N(t)$ for ad libitum fed and feed-restricted (F-R) rats (data from Ross¹³) overlaid on tumor incidence plot for rats treated with 0.15 mg/kg-day DENU.¹⁴ Actual survival would be reduced as tumors form. Survival curves were generated by fitting survival data from Ross¹³ to the function $N(t) = N_0 \exp\{(-q_x/r_x)[e^{r_x t} - 1]\}$, where N_0 is the total number of animals at time 0; q_x is the mortality rate at age 0; and r_x is the acceleration of mortality with time.

tumor induction as late as that shown in Figure 2B might go unobserved for some chemicals. Rozman¹⁵ has shown that differences between chemicals in Dose $\times$ t = Effect behavior is often the result of differences in pharmacokinetic characteristics of the chemicals. Thus, the inability of experimenters to achieve a physiological change and observe the development of an effect within the short lifetime of a test species can be exacerbated by a failure to achieve an equivalent systemic dose between test species. It is therefore important to be able to determine the target tissue dose for test species in subchronic and chronic studies and to ensure that systemic exposures are computed, since the dose provided in the food or other delivery vehicle may not be equally absorbed or may be eliminated more rapidly in different test animal species.

The FDA usually receives results of subchronic and chronic toxicity studies without analyses of tissues that would tell us the target tissue dose. However, we often receive pharmacokinetic study data that include tissue concentrations

Figure 3. Simulated concentrations of an antioxidant (HP-136) in tissues of male and female rats resulting from dosing HP-136 at 10 mg/kg-day. Tissue partition coefficients were based on 96-hour tissue:plasma ratios. Plasma k_e 0.5/day for males and females.

that may allow us to use pharmacokinetic models to estimate target tissue concentration during the conduct of subchronic and chronic studies.

Example: Gender Differences in Tissue Partitioning and Effects

In a recent submission reviewed by FDA, the target tissue of an antioxidant (HP-136) appeared to be the liver. The chemical in question produced hepatic inflammation and necrosis at doses above 50 mg/kg-day in female rats but produced no effects in male rats below 1000 mg/kg-day. No measurements of tissue concentrations of HP-136 were available from the chronic study.

A limited pharmacokinetic study had been conducted, with oral dosing, which showed low levels of gastrointestinal (GI) absorption (<15% of dose) and rapid disappearance of label from the plasma. Only 1 set of tissue samples was taken, at 96 hours postdose. However, these values were pivotal, showing that concentrations in the fat and liver were 30 to 100 times higher than the rapidly disappearing levels in plasma. We used the Liver:Plasma and Fat:Plasma ratios at 96 hours as partition coefficients, incorporated these parameters into a blood-flow limited pharmacokinetic model, and simulated

the dosing conditions of the chronic study (results shown in Figure 3). Female rats were predicted to accumulate very high levels of HP-136 in the liver during the chronic study, while fat was the most accumulative tissue in males—a strong correlation between toxicity and target tissue dose. This outcome suggested that binding to a female-specific cytochrome P450 or other hepatic binding protein was involved.

Without more mechanism-specific information we were not able to determine whether these phenomena were relevant to humans, but the pharmacokinetic models of the rat gave the FDA a stronger argument for reducing exposure levels than we would have had with chronic study results alone. Even if human studies were conducted, information on tissue levels would never have been available from clinical studies. When tissue residue data are available in animals, tissue-blood partition coefficients can be estimated and used with anatomical models to perform extrapolations like that shown in Table 2. Such extrapolations are chiefly useful for checking mass balances in clinical studies but, once validated, can be very useful in evaluating experiment designs by simulation of outcomes.

Since target-tissue concentration data are rarely ever provided with submissions of chronic study results, simple models (see Figure 4) are often used to simulate plasma concentrations, and where more detailed ADME data are available, concentrations in specific tissues may be simulated. In fact, it has been our experience that the results from a good pharmacokinetic study enable us to accurately predict the outcome of multiple dosing studies and chronic dosing studies.

In some cases, a product sponsor may provide the FDA with sufficient pharmacokinetic data and/or a model containing sufficient detail to be called a physiologically based model. A model of this type is what is usually required in order to simulate concentrations of chemicals in specific tissues. While instances of the use of such models by product sponsors are rare, we do encourage the use of such models since the data provided are generally of higher quality and are directed toward answering mechanistic questions rather than giving us qualitative answers that may be much more difficult to extrapolate to humans.

Example: Solubility Limitation on Bioavailability/Dose

An example from another recent submission involved the limitation of chronic study doses by low water solubility of the food additive being tested. Doses implied by levels incorporated into the food of animals on chronic study ranged from a few mg/kg-day to more than 1 g/kg-day.

Although pharmacokinetic studies provided by the sponsor did not extend to the highest doses used in the chronic studies, some analyses of plasma levels were available from chronic studies and indicated that absorption was lower at high doses. When the aqueous solubility for this additive

Table 2. Estimated Distribution of Dose in Human Organs*

Human Organ†	Weight (% BW) (= $V_t/BW \times 100$)	C_t/C_p (from rat)‡ (0.25-6.0 hours)	$(C_t/C_p)V_t/BW$ (%) (0.25-6.0 hours)
Adrenals	0.02	0.241-0.761	0.005-0.0152
Bladder	0.15	1.020-4.637	0.152-0.695
Bone marrow	4.1	0.176-0.641	0.528-1.923
Bone(other)	10.1	N/A	N/A
Brain	1.5-2.0	0.053-0.239	0.106-0.478
Erythrocytes	2.83	0.383	1.084
Eyes	0.03	0.087-0.913	0.002-0.027
Fat	17.8(5.2-21.4)	0.108-0.239	1.922-4.254
Heart	0.30-0.50	0.060	0.018-0.030
Kidneys	0.40	5.152-7.010	2.061-2.804
Liver	1.70-2.6	14.93-13.04	25.38-33.90
Lungs	0.80	0.374-1.26	0.299-1.008
Lymph nodes	0.70	0.231-0.913	0.162-0.639
Muscle	30.0-45.0 (max)	0.098-0.380	2.94-11.40
Pancreas	0.07-0.14	0.394-0.794	0.028-0.111
Prostate	0.02	1.199-1.978	0.024-0.039
Salivary	0.05	0.204-0.902	0.010-0.045
Skin	3.70	0.250-0.576	0.925-2.131
Spleen	0.15-0.26	0.167-0.663	0.025-0.0995
Testes	0.04	0.083-0.456	0.003-0.018
Thymus	0.01	0.100-0.50	0.001-0.005
Thyroid	0.02	0.316-1.195	0.006-0.0239
GI tissue	1.65-1.71	***	***
SUM	76.14%-77.98% BW	N/A	35.88-60.80
Plasma	3.73(6.5% BI, 43% HCT)	1.00	N/A

*BW indicates body weight; GI, gastrointestinal; ***, no reliable measurements available; SUM (= $\Sigma V_t/BW$); Note: mass in tissues/mass in plasma = $\text{Sum}[C_t V_t]/C_p V_p = (BW/V_p) \cdot \text{Sum}[(C_t/C_p)(V_t/BW)]$.

†Human tissue weight (% BW) taken from Brown et al.⁸ and Wasserman et al.¹⁶

‡Tissue/plasma concentration ratios computed from rat study data.

was incorporated into the pharmacokinetic model via a dissolution submodel, the model predictions were very similar to plasma concentrations observed in the chronic study (Figure 5). In this case it could be concluded that the systemic dose achieved by providing ~500 mg/kg-day in the diet would have been essentially equal to that provided by the higher doses. Had any adverse effects been observed, they would likely have been very similar from 500 mg/kg-day up to the top dose, whatever that number might have been. In this case, the models were useful primarily as a means of clarifying experimental results rather than as a means of predicting the outcome of human exposures.

SELECTION OF REPRESENTATIVE SPECIES BY COMPARISON OF METABOLITES, PHARMACOKINETICS

The FDA may receive pharmacokinetic data for more than 1 species for high-volume/high-exposure food additives.

These data are generally accompanied by toxicity studies for the same animal species. When clinical pharmacokinetic studies are available as well, it becomes possible to compare the metabolism and pharmacokinetics between humans and multiple test species. If possible, we would like to select toxicity studies from the species most similar to humans as the basis for predicting the human dose-response (where any unwanted effects occur) and estimating acceptable exposures. We try to use the simplest pharmacokinetic models possible, but in our experience the simplest models that are able to fit major features of GI absorption and plasma elimination curves contain 5 to 7 compartments. These models include a stomach (No. 1), a small intestine (No. 2), one or more blood (plasma) compartments (No. 3-4), and at least 1 tissue compartment (No. 5-6) (see Figure 5). Using such models it has been possible to fit and perform quantitative comparison between test animals and humans with respect to absorption rates, elimination rates, blood:tissue partitioning, and differences in

Figure 4. Pharmacokinetic model used to fit data from oral dosing studies. In this case, formation of the primary metabolite occurs by bacterial metabolism in the large intestine. Other variations of this model have incorporated metabolism in the parent tissue compartment (No. 6), metabolite transport to/from tissue compartment No. 7, and other minor changes.

metabolites produced (Figures 6 and 7). More important than the curve fitting required for estimation of the absorption and elimination parameters is the prediction of systemic exposures in humans and test species by test conditions and proposed food-additive use levels. Small differences between species in absorption and metabolism may combine to yield similar or very different systemic concentra-

Figure 5. Effect of solubility on delivered dose versus systemic dose (maximum plasma concentration achieved). In this case, the test substance was delivered by gavage in a fixed volume. The 150- and 500-mg/kg doses were given in suspension.

Figure 6. Example of the use of model shown in Figure 5 to fit oral dosing data. Parameter values for intestinal absorption ($k_{a(i)}$) and gastric emptying of fluids (E_{01}) from a gavage dosing experiment were combined with estimates for systemic elimination (k_e) and emptying of solids (E_{01}) to simulate plasma levels of parent chemical (neotame) and its metabolite after dosing in food.

tions. Exposures are usually computed as areas under the curve ($AUC = C_i(t) dt$).

In the case of neotame, relatively small differences in basic ADME parameters combine to give a very large difference

Figure 7. Interspecies comparison of (A) quantities of neotame metabolites "A" through "D" produced by each species tested, (B) % of oral dose absorbed (F), plotted on same scale with model-estimated systemic elimination coefficients (k_e) for each species. A significant difference is apparent between humans and rats in each case.

Figure 8. Comparison of the systemic mass of parent compound (neotame) in rat, beagle (DOG), and human subjects following a 20-mg oral dose. Differences are primarily due to extremely rapid plasma esterase activity in rat plasma (k_e 20/h) as compared with human (2.0/h) and dog (5.0/h) esterase activities. Absorption rates differ by approximately a factor of 4 (0.5/h $\rightarrow$ 2.2/h).

in systemic exposure (Figure 8). The normal safety factor approach (~100-fold between humans and most sensitive test species) would not have provided a sufficient margin of safety if any toxicity had been associated with this food additive. Total systemic exposure ($AUC = \int C(t) dt$) to neotame in humans is 600-fold higher than in the rat.

CONCLUSION

The estimation of a “safe” level of human exposure to new food additives has never been an exact procedure and relies on safety studies of varying quality and quantity. The accumulation of historical data on growth and physiology in humans and animals has allowed the development of mathematical models that can provide a reliable and robust means of extrapolating from the available data sets to obtain better dose-response curves and other information we would like to have when performing safety assessments. Without such models, we would often be reduced to hand-waving arguments and piling on additional safety factors in order to achieve “safe” levels of food-additive exposure.

REFERENCES

1. Code of Federal Regulations. 21 CFR 170.3. Washington, DC: US Government Printing Office; 2002.
2. Code of Federal Regulations. 21 CFR 172-177. Washington, DC: US Government Printing Office; 2002.
3. Code of Federal Regulations. 21 CFR 170.22. Washington, DC: US Government Printing Office; 2002.
4. Dourson ML, Stara JF. Regulatory history and experimental support of uncertainty (safety) factors. *Regul Toxicol Pharmacol*. 1983;3:224-238.
5. Roth WL, Young JF. Use of pharmacokinetic data under the FDA's Redbook II guidelines for direct food additives. *Int J Toxicol*. 1998;17:355-381.
6. Roth WL, Freeman RA, Wilson AGE. A physiologically based model for gastrointestinal absorption and excretion of chemicals carried by lipids. *Risk Anal*. 1993;13:531-543.
7. Roth WL, Garthoff LH, Luecke RH, Young JF. Standardized growth models of experimental animals for reconstruction of whole animal pharmacokinetics. *Proceedings of 10th International Conference on Mechanics in Medicine and Biology, March 2-5, 1998*. Honolulu, Hawaii.
8. Brown RP, Delp MD, Lindstedt SL, Rhomberg LR, Beliles RP. Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol Ind Health*. 1997;13:407-484.
9. Deavers S, Huggins RA, Smith EL. Absolute and relative organ weights of the growing beagle. *Growth*. 1972;36:195-208.
10. Luecke RH, Wosilait WD, Young JF. Mathematical representation of organ growth in the human embryo/fetus. *Int J Biomed Comput*. 1995;39:337-347.
11. Luecke RH, Wosilait WD, Young JF. Mathematical analysis for teratogenic sensitivity. *Teratology*. 1997;55:373-380.
12. Murray WW. Biological significance of factors influencing the incidence of mammary cancer in mice. *J Natl Cancer Inst*. 1964;34:21-41.
13. Ross M. Length of life and nutrition in the rat. *J Nutr*. 1961;75:197-210.
14. Druckrey V, Schildbach A, Schmähel D. Quantitative analyse der carcinogenen wirkung von diäthylnitrosamin. *Arzneimittel Forsch*. 1963;13:841-851.
15. Rozman KK. Delayed acute toxicity of 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin (HpCDD), after oral administration, obeys Haber's rule of inhalation toxicology. *Toxicol Sci*. 1999;49:102-109.
16. Wasserman LR, Yoh TF, Rashkoff IA. Blood volumes in human subjects as determined by P^{32} labeling of erythrocytes. *J Lab Clin Med*. 1951;37:342-352.