Comments on

Acrylamide Review Panel Draft Report on *"USEPA Toxicological Review of Acrylamide" In Support of Summary Information on the Integrated Risk Information System (IRIS) December 2007"*

Submitted by

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Submitted to the Science Advisory Board
US Environmental Protection Agency

On Behalf of

Grocery Manufacturers Association
Washington, DC

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Comments on
Acrylamide Review Panel Report on
"USEPA Toxicological Review of Acrylamide in Support of Summary Information on the Integrated Risk Information System (IRIS) December 2007"

INTRODUCTION AND SUMMARY —

On behalf of the Grocery Manufacturers Association (GMA), *The Sapphire Group, Inc.*, offers to USEPA's SAB comments on the draft report prepared by the SAB's Acrylamide Review Panel (ARP). This ARP report addresses USEPA's draft Toxicological Review of Acrylamide. We applaud the efforts put forth by the Agency's ARP in its review. That notwithstanding, we elaborate herein on some advanced information that we recommend be taken into account by the ARP before final issuance of its report and ultimately by the group within the Agency responsible for advancing the draft Toxicological Review of Acrylamide to its next level.

On 2 April of this year, we provided assorted, detailed comments to both the Agency and the ARP about the Agency's draft Toxicological Review of Acrylamide. While some of the concerns we raised have been addressed, others appear to remain outstanding. Since that time, we have had occasion to study in considerable detail the scientific underpinnings of a central element of the Agency's draft, most notably the physiologically-based toxicokinetic (PBTK) modeling employed in the Agency draft. We hope to demonstrate clearly that the ARP has not addressed some important limitations of the Agency draft with particular reference to the PBTK modeling and factors influencing the cancer potency for ingested acrylamide. We request the SAB to take particular note of the fact of acrylamide's inadvertent formation in cooked foods, resulting in it being of potential public health importance not only in the United States but also around the world. We recognize that USEPA's regulatory purview extends only to environmental exposures such as drinking water and not to exposures in the workplace or to foods and some consumer products. Although USEPA has no responsibility to accurately assess acrylamide in food, the Agency must recognize that its toxicological review of acrylamide may become a reference for food regulators and risk managers. It is therefore incumbent on USEPA to make this review as robust as possible, including a clear discussion of alternatives and uncertainties in the assessment.

We ask, therefore, that the SAB commission the ARP to reconsider elements of its report before making its report final. To do so, we realize, may require some modification to the ARP's original charge.

The bases of our recommendation are as follows.

1. The PBTK model used by the Agency is flawed. ARP correctly acknowledged the central importance of the Agency's PBTK model ("recalibrated Kirman model") for acrylamide to its estimate of the noncancer RfD (based on neurotoxicity) and of the cancer potency. The recalibrated model summarized in the Agency's draft Toxicological Review of Acrylamide is based on (a) the model code that seeks to integrate assorted data into a dynamic representation of the metabolism and biological interactions of acrylamide and its primary metabolite (glycidamide) in the body of the rat; and (b) the documentation describing the information used and the functionality of the model with sufficient detail so as to impart transparency to third-party reviewers and users of the recalibrated acrylamide model. While the ARP identified several shortcomings of the recalibrated Kirman model, it was unable to ascertain the serious flaws in the model.

According to its draft report, the ARP either did not have access to the model code and its documentation or had insufficient resources to examine them in detail. Thus, ARP could not have examined or critiqued in detail the model structure to determine its integrity, completeness, or validity. We obtained these documents, and our kineticists have analyzed them closely. Our findings about the acrylamide PBTK model include:

- The model has not been subjected to the validation steps considered necessary by the community of PBTK model developers as well as Agency guidelines. Without the use of these rigorous approaches, it is unclear if the model's results for acrylamide are indeed valid.

- The model is out of date since it was completed in 2005 and much relevant data have been published since that time.

- Some parameters used in the model are physiologically impossible, leading to misstatements of internal doses.

- The model documentation lacks sufficient transparency, making it difficult to understand the model and replicate it.

- The model code *The Sapphire Group* received from the Agency's contractor was not operational due to an original programming error. That information has been conveyed to its author, who has volunteered to address the matter

with the Agency. It is unclear whether the Agency had a different version of the model code when it prepared it draft assessment.

The details of our findings are found herein in **Appendix A**. **We ask that the SAB consider these observations before completing its report.**

2. Although several limitations of the recalibrated Kirman model, some of which have been identified by the ARP, were substantial, the charge to the ARP did not encompass reconstruction of the reconstructed model to overcome those deficiencies. Due to our experience with the original Kirman et al. PBTK model for acrylamide and our understanding of the kinetic data on acrylamide, we have undertaken to produce an update of the Kirman model employing (a) all the relevant and most up-to-date scientific data about the compound's kinetic behavior and (b) a validation step. The updated model is scheduled to be completed by the end of 2008, at which time it will be submitted for publication in a peer-reviewed journal. It can then be used by the scientific community to develop internal doses of acrylamide that would serve as inputs into estimation of RfD and cancer potency factors. A summary of our progress is described in **Appendix B**. **SAB should ask ARP to await that updated model and consider it in the Agency's update of its draft Toxicological Review of Acrylamide.**

3. The ARP current draft refers to only a small fraction of the irreversible binding or sequestration detoxification mechanisms (both capacities and rates) present in humans into its estimate of cancer potency and risk-specific doses. Detoxification is a means for the body protect itself from what might otherwise be potentially dangerous exposures to chemicals foreign to the body (details were provided in our earlier comments to ARP and the Agency). Particularly noteworthy is the fact that humans have a greater abundance of these defense mechanisms than do the rats used to test experimentally for the carcinogenicity of acrylamide. The existence of effective detoxification mechanisms is to be expected on biological grounds because hundreds of naturally-occurring toxicants are intrinsic to the diet. Compounds that form naturally with heating, like acrylamide, have been in the diet since the advent of cooking.

This one factor alone may reduce human cancer risk estimates from ingested acrylamide; however, the ARP report does not address with any emphasis the consequences of these binding/sequestration processes for estimating cancer potency at doses experienced by humans in tap water or the diet (see our earlier submission for details). We have completed and submitted for peer-reviewed publication a manuscript that details the role of detoxification of acrylamide and its primary metabolite glycidamide. Comments from reviewers were received recently with an indication that once the suggested changes are incorporated, the manuscript will be accepted for publication, with publication in two to four

months. **We ask the SAB to defer finalizing its ARP report until the ARP can consider this publication and conduct a more detailed analysis of detoxification of acrylamide in the diet.**

4. The ARP draft report deals only peripherally with alternative shapes of the dose-response curve for acrylamide carcinogenicity. **The ARP should direct USEPA to include a non-linear approach for extrapolating to low doses in the range experienced by humans in addition to the linear default approach used by the USEPA for any pertinent cancer endpoints. This situation is particularly important when the lower bound cancer risk may be zero, as is the case with acrylamide.** Unless these steps are taken, decision makers and others will not be fully informed of the range of possible cancer potency factors for — and potential cancer risks of — acrylamide exposures for humans.

The ARP review should promote balance and perspective in the Agency's characterization of acrylamide's cancer risk. First, the ARP should not dismiss out of hand the evidence that has been submitted to the ARP and the Agency that supports non-genotoxic MoA, or has been submitted to the ARP and the Agency to support at least a mixed MoA. Even if acrylamide were assumed to be causing cancer through a genotoxic mode-of-action (as yet an unproven hypothesis), it is important to present quantitatively and transparently the impact of both possibilities on the estimated potency. In other words, the ARP report should say that, depending on whether acrylamide is assumed to be acting through a genotoxic or non-genotoxic mode-of-action, the cancer potency factor would be either "X" or "Y." Furthermore, when using the linear assumption, ARP should direct the Agency to report the lower-bound on cancer potency and risk as well as the upper-bound. Such an approach would be appropriate even if genotoxicity were the sole MoA. Precedents exist for genotoxic carcinogens to have non-linear dose-response relationships. [Relevant documentation has been provided to ARP and the Agency in our earlier submission.]

Based on the above, it seems prudent that the USEPA conduct an RfD-type cancer assessment (*i.e.*, NOAEL or BMDL and Uncertainty Factors), in addition to the default linear approach presently described, in the next version of the Agency's document. Another appropriate methodology would be that called the Margin of Exposure (MoE), by which one compares a human equivalent NOAEL to known or anticipated human doses. Another approach worthy of serious consideration by ARP is the formulation of a biologically-based model that incorporates mixed MoA for low-dose extrapolation of cancer incidence in rats and subsequent application to humans.

5. The ARP draft is incomplete with regard to judging the relevance to humans of the tumors observed in acrylamide-exposed rodents. A **Mode-of-Action/Human Relevance Framework** exists to do so in considerable detail and with much reliability and transparency. USEPA has accepted this Framework as part of its assessment process (as mentioned in our

previously submitted comments). In this instance, the Agency has applied this Framework to an insufficient extent to acrylamide's Toxicological Review. Were it to apply the Framework more completely, it would find that all rat tumors are not relevant to humans. Missing from USEPA's draft are (a) taking into proper account kinetic and dynamic factors, (b) plausibility of MoA for humans, and (c) concordance analysis of animal and human responses (notwithstanding USEPA's default view that no such concordance need exist). Considering that acrylamide is intrinsic to the diet and cannot be completely eliminated, such an analysis would prove to be essential understanding whether there may be any public health implications and informing risk management decisions. **The ARP should address this matter in sufficient detail to demonstrate the value of this approach in the next iteration of the Agency's Toxicological Review of Acrylamide.**

We wish to provide the following additional comments related to the ARP Review.

1. We agree with the ARP's conclusion that the Agency draft treatment of acrylamide's neurotoxic properties demonstrated a sound understanding of the underlying science, an appropriate application of its methodology in generating a draft RfD, and a clear and understandable rationale of how it developed the draft RfD. We agree that the proposed RfD based on neurotoxicity is amply protective of public health, and suggest that USEPA consider the prospect that protecting against neurotoxicity may also protect against cancer (when the cancer potency is properly classified as non-linear, as noted in our comments).

2. The ARP draft report does not recognize the restricted value of USEPA's approach to the time-to-response model, and recommend that the Agency address early mortality by methods other than that employed in the Agency draft.

3. Some dose-response data from the Johnson et al. (1986) study may be useful for estimating the cancer potency of acrylamide and should be considered for inclusion in the quantitative assessment. The draft ARP report is silent on this point but should include an indication that the data may have some utility.

4. We agree with ARP that AUC for glycidamide is the best dose measure for a genotoxic MoA. We recommend, however, that ARP indicate that to the extent that a non-genotoxic MoA can be justified, use of AUC acrylamide could be supported for a non-linear as well as linear assessment.

5. ARP should inform the Agency that its use of a time-to-response model for acrylamide carcinogenicity is unwarranted. The model for male rats does not appear to be supported for tunica vaginalis mesothelioma data. On a practical level, the multistage-Weibull time-to-response model used by the Agency in its dose-response assessment for the carcinogenic effects of acrylamide is no longer available or supported, thereby adversely affecting the transparency and reproducibility of USEPA's assessment.

6. The ARP draft review should inform USEPA to present not only central tendency and upper-bound estimates of cancer potency for acrylamide but also a lower bound estimate, as indicated by the Agency's current carcinogen assessment.

7. Because acrylamide has been recently reported as being present in widely consumed foods, USEPA's judgments about human safety and risks may well have impacts in our society and globally that greatly transcend its legislated mandates. The SAB and the Agency should recognize the existence of major ongoing toxicological studies (sponsored by the National Toxicology Program) whose findings, expected by the end of 2009, may change, perhaps greatly, the USEPA's estimates of cancer potency of ingested acrylamide.

Therefore, the SAB and the Agency should proceed with particular caution in finalizing its Toxicological Review of Acrylamide and in the formulation of its IRIS documentation for acrylamide. **We ask, therefore, that the SAB recommend that the Agency defer issuance of its final Toxicological Review of Acrylamide and its IRIS documentation until the peer-reviewed NTP data become publicly available.**

APPENDIX A

Findings from a Detailed Review of USEPA's
PBTK Acrylamide (AA) Modeling Documentation

Prepared for
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INTRODUCTION

USEPA released a draft Toxicological Review in December 2007 in which toxicity reference values (a chronic Reference Dose, chronic Reference Concentration, cancer oral slope factor, and cancer inhalation unit risk) were derived for acrylamide (AA) for the Integrated Risk Information System (IRIS) (USEPA, 2007). An important part of the toxicity reference value derivation process was the use of a physiologically based toxicokinetic (PBTK) model for interspecies and route-to-route extrapolation.

USEPA had identified the PBTK model of Kirman et al. (2003) as being of potential value in deriving the AA toxicity reference values and employed a contractor to modify ("recalibrate") and apply it for the IRIS AA draft to calculate Human Equivalent Doses for noncancer and cancer. The contractors' efforts were completed in 2006, and the results of that modeling were incorporated in the Agency's draft IRIS document for AA.

A Science Advisory Board (SAB) was convened by USEPA to provide a peer review of the AA Toxicological Review. Several charge questions pertaining to PBTK modeling were put before the SAB for their discussion.

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Charge Question 8:

Please comment on whether the documentation for the recalibrated Kirman et al. (2003) PBTK model development, evaluation, and use in the assessment is sufficient to determine if the model was adequately developed and adequate for its intended use in the assessment. Please comment on the use of the PBTK model in the assessment, e.g., are the model structure and parameter estimates scientifically supportable?

Charge Question 9:

Do you agree with the conclusion that the recalibrated Kirman model is the best for deriving toxicity values?

Charge Question 11:

Is the recalibrated PBTK model appropriate to use for route-route extrapolation?

The Sapphire Group, Inc. reviewed the IRIS AA draft and evaluated the PBTK model used by USEPA and its application in the toxicity reference value derivations. Particular concerns were the transparency of the model development/calibration and validation processes and the failure to test whether or not the AA model used by USEPA was consistent with recently developed data (The Sapphire Group, 2008). The Sapphire Group's comments were provided to the SAB by GMA. Some of the concerns could not be fully evaluated because we did not have access to a copy of the model and the documentation provided by USEPA was limited. Subsequently we were provided access to electronic copies of the model and additional documentation. The assessment in this current report therefore reflects our understanding based on this additional information.

PROCESS AND APPROACH

Following the SAB public meeting, *The Sapphire Group* has conducted a follow-up review for which the contractor to USEPA provided copies of the spreadsheets and reports which documented the analyses used in the USEPA assessment. Access to these documents allowed us to investigate some of our previously identified concerns.

We reviewed the spreadsheets and reports to identify which of them documented the versions of the model that USEPA ultimately used in the IRIS draft Toxicological Review. We reviewed the equations in these spreadsheets to confirm that AA and its metabolite glycidamide were appropriately accounted for throughout the computations. For example, if AA is eliminated from the liver by metabolism (subtracted in the liver AA mass balance equation), a molar equivalent amount of glycidamide must be created and added to a mass balance equation for glycidamide in the liver. We also compared the model parameter values

in the key spreadsheets to those in the contractor's reports to the USEPA and USEPA's IRIS document.

FINDINGS

The findings below reflect those findings related to PBTK modeling that were identified in our previous effort (The Sapphire Group, 2008), updated to reflect additional insights in light of the additional items which have subsequently been reviewed.

1. The recalibrated rat PBTK model lacked validation at the time it was completed (e.g., Doerge et al., 2005 a,b).

In the recalibration, the contractor emphasized fit to cumulative measures (end-of-exposure hemoglobin adduct levels and 24 hr urinary excretion). The Doerge et al. (2005a,b) data include measurements of AA and glycidamide in serum at several time points for rats exposed to AA or glycidamide by iv injection, gavage, or dietary exposure. Combinations of model parameter values that produced adequate simulations of metrics at the end of the simulation may not necessarily adequately describe how the concentrations of AA and glycidamide change during the exposure. That is, the model simulations may have given accurate predictions of the original data set for the wrong reasons, and this may not be apparent until the model is tested against a different type of data. Since PBTK models are frequently applied to dosing scenarios that are dissimilar to those used in the studies considered during model development (e.g., extrapolation to lower doses, repeated dosing), it is important to compare the model to an extensive, diverse data set, if such data are available. USEPA failed to fully validate their AA model in the draft Toxicological Review.

2. The USEPA PBTK documents lack transparency. While the absence of transparency may not change the results of USEPA's analysis or their toxicity reference values, it limits understanding and replication of USEPA's findings, thereby impairing the credibility of the IRIS documentation.

Physiologically impossible parameter values were listed in the limited model documentation provided by USEPA (USEPA, 2007). The human liver volume listed in Table E-8 is 0.183, which happens to be the same as the fractional liver blood flow in this table. Likewise, the fractional tissue blood flow and fractional tissue volumes in Table E-8 are also identical (0.8842). These values are problematic as the sum of tissue volumes and blood flow to tissues in humans are greater than 100%, a physical impossibility. It is surprising that no one identified this error during the preparation, internal review, and interagency review of the IRIS draft document. These same values are found in the report provided to USEPA by the

contractor, but these are not the values that were actually used in the spreadsheet calculations.

A further discrepancy between the model documentation is that most of the metabolism parameters were not correctly reported in the USEPA Toxicological Review (2007) or the contractor reports. The spreadsheet calculations typically included "adjustment factors" that were apparently manipulated by the user to achieve optimal fits to the experimental data. The reports neglected to include these adjustment factors in the calculation of the final best-fit model parameters, and as a result, the parameter values actually used in the model calculations differ by as much as 3.6-fold from the reported values. Without inspecting the spreadsheet, it is unlikely that these reporting errors could have been identified.

We have brought these findings to the contractor's attention, who upon review of the spreadsheets and reports, concurred with *The Sapphire Group*'s conclusions.

The calculations in Appendix Tables E-9 (human model predictions) and Table E-10 and E-11 (AUCs for AA and glycidamide for various AA doses via a drinking water exposure or inhalation) values were calculated using the same parameter set. Thus there was no error introduced between human model development and application of those model parameters in the two human exposure scenarios.

3. *The Sapphire Group, Inc.* found that the spreadsheets do not accurately recreate the Kirman et al. (2003) rat model.

In Appendix A of Kirman et al. (2003), reaction rates are clearly specified as being expressed as function of liver venous blood concentrations. In the USEPA recalibrated model, these same rates are in terms of the concentration in liver tissue. Thus, the IRIS simulations of the Kirman et al. (2003) model as originally calibrated (e.g., Table E-3 in Appendix E of the IRIS document) are in error. The error may not be large, however, since the liver tissue:blood partition coefficient was 0.83. As a result, in the USEPA model calculations, the AA metabolism rate was underestimated by as much as 17 percent. However, since USEPA used an update of the rat model in their risk value derivations, any error in describing the Kirman model does not propagate into the quantitative aspects of the assessment itself.

4. The human PBTK model has limitations that could be explored using additional data and by conducting sensitivity analyses which USEPA has either not conducted or not reported.

The USEPA's contractor used a very limited data set to parameterize the human model, and the data of Fuhr et al. (2006) and Kopp and Dekant (2008) (abstract only) were not used. The impact of the omission of these data cannot be determined until we know whether or not USEPA's model output is consistent with the data of Fuhr et al. [Comparison to the data of

Kopp and Dekant would also be of value, though no firm conclusions should be drawn until a full, peer-reviewed report of the data is available.] When faced with highly uncertain parameter values with limited validation, USEPA should conduct sensitivity analyses of the dose metrics used in the assessment. Without this information, model confidence cannot be assigned, and thus the confidence in the quantitative aspect of the assessment cannot be assessed.

CONCLUSIONS AND RECOMMENDATIONS

We conclude that the USEPA has used a model that was out-of-date by the time their assessment was completed. At a minimum, USEPA should have conducted additional simulations that would test whether their model was or was not consistent with the new data, and either revised their assessment or provided appropriate caveats. New or revised rat and human models should be developed and the assessment should be updated accordingly. Furthermore, the public and the reviewers of an updated assessment should be provided with sufficient, accurately-prepared documentation of the PBTK modeling so that a practitioner in the field could accurately reproduce the model and the simulations in the assessment.

APPENDIX B

Progress report for updating the acrylamide-glycidamide PBPK model

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INTRODUCTION

Based on the findings of our review of the USEPA's draft Toxicological Review for acrylamide (AA) and the available literature on the disposition of AA and its key metabolites, we concluded that the development of an updated PBPK model for AA was needed for a state-of-the science risk assessment of AA. The following report provides an overview of the work completed thus far toward the development of an updated PBPK model for AA and its metabolites in the rat. Information on planned subsequent activities is also provided. As model development is typically an iterative activity, there may be further changes to the model structure and changes in optimization/parameterization strategies prior to the completion of this effort. Human modeling efforts will be initiated once the rat model is deemed satisfactory. A more detailed description of the final model structure, the process for deriving the parameters for the model, and strengths and limitations of the model will be provided in the draft manuscript which is the intended outcome of this effort.

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MODEL STRUCTURE

It was decided within our project team that alterations to the Kirman et al. (2003) model structure would be of value, in consideration of data that have come available since the publication. We plan to implement the following changes/additions to the model structure:

- The 2003 model consisted of blood (plasma plus erythrocytes), liver, and "tissue" compartments for AA and glycidamide (GA). The "tissue" compartment will subdivided into compartments corresponding to the brain, kidneys, slowly perfused (muscle and skin), and other perfused tissues.

- Urinary elimination of GA from the kidney will be added to the model.

- Simple one-compartment model descriptions will be included for the major urinary metabolites (the mercapturic acids derived from conjugation of AA and GA with GSH, and glycidol) rather than direct excretion.

- GSH conjugation of AA and GA will be described as saturable with respect to GSH concentration, first order with respect to AA and GA concentration.

- Liver GSH synthesis and depletion will be incorporated into the model.

IDENTIFICATION OF DATA SETS AND ORGANIZATION OF DATA

The data used in developing the Kirman et al. (2003) and Young et al. (2007) models, as well as additional data identified via literature review, have been reviewed, digitized (if necessary), and organized into Excel spreadsheets. The spreadsheets will facilitate the transfer of the data into the PBPK modeling program (ACSLx) as well as for export into software producing publication-quality graphics (GraphPad Prism).

PRELIMINARY PARAMETER ESTIMATES

Physiological/anatomical parameter values (e.g., blood flows, tissue volumes) have been identified from the literature. Protein turnover rates for some tissues (liver and muscle) have been identified from the literature.

Tissue:blood partition coefficients for AA and GA have been estimated from the data of Doerge et al. (2005a). A single set of partition coefficients for male and female animals will be used.

Initial estimates for metabolism parameters will be derived from a variety of sources. The parameters determined by Young et al. (2007) for iv kinetics of AA and GA in male F344 rats will be converted into body weight-normalized first-order reaction rate estimates. Km estimates will be taken from other sources (e.g., Kirman model, Walker et al., 2007 model, *in vitro* studies). If reported *in vivo* serum and tissue levels are sufficiently high, metabolism will be described as saturable; otherwise, first order reaction rate equations will be used. Because Young et al. (2007) did not include hydrolysis of GA in their model, the Kirman et al. (2003) values for GA hydrolysis will be used in the initial estimates.

Oral absorption rate estimates will be taken from Young et al. (2007). Blood and tissue protein binding rates from the Kirman et al. (2003) model will be retained as initial estimates.

OPTIMIZATION/PARAMETER ESTIMATION

In general, the approach to parameter estimation will be similar to that employed by Young et al. (2007). We will focus on the F344 rat data and start with the GA iv studies (GA serum time course). Because we will include a metabolic pathway not included by Young et al. (2007), other parameters will need to be adjusted from those determined by Young et al. (2007) to maintain consistency between the model predictions and the data. Next, we will consider the AA iv studies (AA and GA serum time courses and urinary metabolites). Unlike Young et al. (2007), we intend to identify metabolism parameter values that adequately describe all the data for male rats rather than optimizing parameter values for each data set separately. We will consider whether the male-female parameter differences in the Young et al. (2007) optimizations reflect a true gender difference or simply a body weight difference or experimental variability. The Edwards (1975) data were not used in any previous modeling efforts, but the GSH depletion data in this study may provide a useful "reality check" as to the total flux through the GSH-conjugation pathways, though this study was not conducted in F344 rats. The 8-24 hr data in Miller et al. (1982) study and Ramsey et al. (1982) study will provide a reality check for the protein binding rates, while the longer term data provide insights into the protein turnover in the blood and tissues. Once the iv data are reasonably well simulated, absorption rates for ip, gavage, and dietary administration of AA and GA will be estimated from the available blood, tissue, and urinary data. Metabolic parameters will be revised as necessary to provide adequate fit to all of the F344 rat data, or "outlier" data sets may potentially be identified. The parameters derived for F344 rats will be used to simulate kinetic studies conducted in other strains–Long-Evans (Crofton et al., 1996; Raymer et al.,1993), Sprague-Dawley (Kadry et al., 1999; Barber et al., 2001), and Wistar (Sanchez et al., 2008). Based on the findings in other strains, we may consider re-optimizing key parameters that can reasonably be anticipated to vary among strains.

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