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AIR FORCE RESEARCH LABORATORY
WRIGHT-PATTERSON AIR FORCE BASE OHIO 45433

28 June 2000

MEMORANDUM FOR US EPA
NCEA (MD-52)
RTP, NC 27711
ATTN: ANNIE M. JARABEK

FROM: Jeffrey W. Fisher, Ph.D.
AFRL/HEST
Operational Toxicology Branch
2856 G St, Bldg 79
Wright-Patterson AFB, OH 45433-7400

SUBJECT: Consultative Letter, AFRL-HE-WP-CL-2000-0035, Physiological Model for Inhibition of Thyroidal Uptake of Iodide by Perchlorate in the Rat.

1. The Operational Toxicology Branch performed several pharmacokinetic intravenous dosing studies with adult male Sprague-Dawley rats using cold and radiolabeled perchlorate and radiolabeled iodide. The intent of these studies was to obtain information on the uptake, disposition and clearance of perchlorate in the rat and examine its influence on the uptake of radiolabeled iodide into the thyroid gland. These data sets are the basis for the development and validation of physiologically based pharmacokinetic (PBPK) models for perchlorate and ^{125}I with carrier iodide in the adult male rat that describe inhibition of thyroidal uptake of iodide (see attachment).

2. Another modeling effort is ongoing to develop a biologically based physiological (BBP) model for thyroid hormone homeostasis. The BBP modeling effort is a feedback model that accounts for the regulation of the thyroid by TSH and regulation of TSH production by T_4. Components of this model include endogenous iodide, iodination of thyroglobulin (Tg), perchlorate, thyroid stimulating hormone (TSH) and total thyroxine (T_4). Up regulation of the thyroid (positive feedback) occurs by increasing TSH levels in the blood. This results in increases in the rate of iodination of Tg, increases in the rate of secretion of T_4 and increases in the rate of uptake of iodide into the thyroid gland. Up regulation of TSH production (negative feedback) occurs by decreases in T_4 levels in the blood.

3. Questions about this report should be directed to Kyung Yu, Ph.D., AFRL/HEST, at 937-255-5150 ext. 3184.

JEFFREY W. FISHER, Ph.D.
Senior Scientist
AFRL/HEST

Attachment: Physiological Model for Inhibition of Thyroidal Uptake of Iodide by Perchlorate in the Rat

1st Ind, AFRL/HEST 28 June 2000

MEMORANDUM FOR US EPA
ATTN: MS. ANNIE JARABEK

This letter report has been coordinated at the branch level and is approved for release.

DAVID R. MATTIE, Ph.D.
Acting Chief
Operational Toxicology Branch
Human Effectiveness Directorate

Physiological Model for Inhibition of Thyroidal Uptake of Iodide by Perchlorate in the Rat

[1]Jeffrey W. Fisher, [2]Latha Narayanan, [3]Richard J. Godfrey, [1]Paula N. Todd, [1]Timothy A. Bausman, [1]Susan M. Young, [4]Teresa R. Sterner, [1]David R. Mattie and [1]Kyung O. Yu

[1]AFRL/HEST
2856 G St, Bldg 79
Wright-Patterson AFB, OH 45433-7400

[2]GEO-CENTERS
2856 G St, Bldg 79
Wright-Patterson AFB, OH 45433-7400

[3]ManTech Environmental Technologies, Inc.
P.O. Box 31009
Dayton, OH 45437-0009

[4]Operational Technologies Corporation
1370 N. Fairfield Rd, Ste. A
Dayton, OH 45432

June 28, 2000

INTRODUCTION

Very little animal or human pharmacokinetic data for perchlorate exist in the published literature. However, perchlorate is used extensively in thyroid research because of its ability to mimic iodide and competitively inhibit the uptake of iodide in the thyroid gland. Perchlorate is also used as a clinical diagnostic tool for assessing thyroid function (perchlorate discharge test) (Wolff, 1998). Two published animal pharmacokinetic studies are available with tissue or serum time course data for ^{36}Cl-radiolabeled perchlorate. Chow and Woodbury (1970) dosed rats and guinea pigs by intraperitoneal *(ip)* injection and collected pharmacokinetic data for $^{36}ClO_4$ in plasma and thyroid gland over a 4-hour period. Additional data were collected for $^{36}ClO_4$ in the thyroid over a 24-hour period. Goldman and Stanbury (1973) administered $^{36}ClO_4$ by *ip* injection to rats maintained on a low iodide diet and collected urine and serum over a 96-hour period. The authors report that the urinary and serum half-lives for $^{36}ClO_4$ were both about 22 hours. Administered $^{36}ClO_4$ was excreted in urine with little or no metabolism.

Several anions (e.g., bromide, astatide, perrhenate, pertechnetate, nitrate and perchlorate) in addition to iodide (Chow *et al.*, 1969), are concentrated in the thyroid gland by an active transport mechanism known as the sodium iodide symporter (NIS) (Wolff, 1998). These ions are not sequestered to the extent of iodide. Anbar *et al.* (1959) was first to report on the uptake of $^{36}ClO_4$ in the thyroid. Chow *et al.* (1969) repeated this earlier work and dosed male Sprague Dawley rats and guinea pigs with $^{36}ClO_4$ (0.69-14 mg/kg) under control and TSH pretreatment conditions. TSH pretreatment increased the uptake of perchlorate in the thyroid compared to controls. The thyroid:plasma $^{36}ClO_4$ concentration ratio was inversely related to dose, suggesting that the transport mechanism (NIS) was saturable and, at the higher doses (above 3 mg/kg), perchlorate was probably inhibiting its own uptake into the thyroid. The inhibitory effect of excess serum iodide on the thyroidal uptake of iodide is called the Wolff-Chaikoff effect (Wolff and Chaikoff, 1948).

Several studies are published on the distribution of perchlorate in tissues. Durand (1938), Anbar *et al.* (1959), Chow *et al.* (1969) and Pena *et al.* (1976) performed distribution studies with $^{36}ClO_4$ in laboratory animals. $^{36}ClO_4$ was found above plasma concentrations in ovaries, salivary gland and adrenal gland. Halmi *et al.* (1956) demonstrated that large doses of perchlorate lowered thyroid and gastric juice iodide concentrations in rats. Pena *et al.* (1976) found that perchlorate inhibited uptake of iodide in the eggs of hens dosed with perchlorate. These distribution studies support the contention that the NIS is present in tissues other than the thyroid. Molecular studies recently confirmed that many tissues (GI tract, thyroid, adrenal gland, ovary, testis, heart, lung, mammary gland, pituitary gland and thymus) contain NIS as measured by mRNA levels (Spitzweg *et al.*, 1998).

^{125}I-radiolabeled iodide studies in animals are available in the literature. These studies demonstrate the accumulation of ^{125}I in the thyroid and the influence of anions, such as perchlorate, on accumulation of radiolabeled iodide in the thyroid. Extrathyroidal distribution studies with ^{125}I are also available. There are few time-course studies published in the literature for radiolabeled iodide. Perlman *et al.* (1941) investigated the time course kinetics of ^{125}I in the

kidney, skin, testes, liver, adrenals, muscle and brain of the rabbit and expressed the uptake and clearance of radiolabeled iodide in each tissue as percent of dose. Halmi *et al.* (1956) examined distribution of ^{125}I in several tissues at 1 to 1.5, 4 to 4.5 and 24 to 24.5 hours after subcutaneous injection of carrier free ^{125}I into male Sprague-Dawley rats. Organ:serum ratios below 1 were reported for all tissues, excluding thyroid, with the exception of the stomach wall and gastric juice. Administration of 100 mg/animal of perchlorate diminished the uptake of ^{125}I in the stomach wall and gastric juice.

The goal of this project was to develop a PBPK model for perchlorate with the ability to simulate plasma or serum concentrations of perchlorate after intravenous (*iv)* administration of a wide range of doses in the naïve rat. The working hypothesis is that the concentration of perchlorate that perfuses the thyroid gland is directly related to the degree of inhibition of uptake of ^{125}I into the thyroid gland. Administration of a trace amount of radiolabeled iodide was used to mimic the anticipated effect of perchlorate on the uptake of endogenous iodide. Model predictions of ^{125}I (with carrier) uptake into the thyroid as a function of perchlorate dose are described using a competitive inhibition equation.

METHODS

A brief description of the kinetic studies designed to aid in PBPK model development for perchlorate and radiolabeled iodide is found below. A ^{36}Cl-perchlorate *iv* time course study was conducted in which several tissues and organs were collected for analysis of ^{36}Cl-perchlorate. This data set was used for model development for perchlorate. All other perchlorate *iv* dosing studies represent model predictions of perchlorate with no changes in model parameters.

Endogenous iodide was not measured in the rats. Naïve rats were dosed intravenously with a very small dose of radiolabeled iodide mixed with cold iodide in saline. The PBPK model parameters that describe mass transfer of iodide represent carrier free ^{125}I mixed with cold iodide in saline (33 μg/kg) and do not represent mass transfer constants for endogenous iodide. Nevertheless, the impact of perchlorate on the kinetic behavior of iodide in the thyroid can be evaluated by monitoring trace amounts of radiolabeled iodide in the body. Based on previous research (Wolff and Chaikoff, 1948), administration of a small amount of ^{125}I was thought to mix with the endogenous pool of free iodide and mimic the behavior of the endogenous pool of free iodide. To help verify this assumption, rats were intravenously dosed with two doses of ^{125}I. Rats were dosed with either carrier free ^{125}I (2.6 ng/kg) or carrier free ^{125}I mixed with cold iodide in saline (33 μg/kg). Both doses provided similar uptake kinetics in the thyroid. The percent of bound iodine in the thyroid of the carrier free dose at 15, 30, 60 and 120 min after dosing was 84, 90, 92 and 92%, respectively and in the iodide with carrier dose, 79, 79, 93 and 94%, respectively. The latter dose was selected to evaluate the effects of perchlorate on uptake of radiolabeled iodide into the thyroid (Yu *et al.*, 2000).

Experimental

Radiolabeled Perchlorate Administration

A time-course kinetic study with $^{36}ClO_4$ was conducted to construct a physiologically based pharmacokinetic (PBPK) model for perchlorate in the adult male rat. Naïve male Sprague-Dawley rats (300 ± 20 g) were dosed once by intravenous tail vein injection with 3.3 mg/kg radiolabeled ammonium perchlorate ($NH_4{}^{36}ClO_4$, specific activity 493 mCi/mole from New England Nuclear, North Billerica, MA). Each rat received less than 6 μCi. Due to the low specific activity, a smaller dosing level could not be achieved. Rats were euthanized (n=6 per time point) by CO_2 asphyxiation at 0.5, 6, 12, 24,32 and 48 hours after dosing. The thyroid, intestinal tract, intestinal tract contents, muscle, skin, liver, kidney, spleen, bladder, plasma and red blood cells were harvested from the rats and stored at -70° C until analysis of radiolabeled perchlorate. Rats for 12, 24, 32 and 48 hours time points were placed individually in metabolism cages for urine collection. Metabolism cages were washed with 500 μL de-ionized water. Urine and cage wash samples were stored under the same conditions until analysis.

Analysis of Radiolabeled Perchlorate in Fluids and Tissues

One mL of a mixture of Soluene-350 and isopropyl alcohol (1:1) was added to 0.1 mL of blood. The mixture was shaken at 50°C until clear and then cooled to room temperature. Half of a mL hydrogen peroxide (30%) was added dropwise with swirling and the mixture was left to stand for 20 minutes at ambient temperature. Fourteen mL of Hionic Fluor was added and radioactivity of the reaction mixture was measured by a liquid scintillation counter (LSC, Packard, Meriden, CT).

Samples of liver, kidney, spleen, skin or muscle (80 ± 20 mg tissue), both thyroid lobes or whole bladder were mixed with 1 mL of Soluene-350. The mixture was shaken at 50°C until clear. Upon cooling, the remainder of the procedure for blood was used for analysis.

Fourteen mL of Ultima Gold plus 0.1 mL plasma, 0.05 mL urine or 0.5 mL cage wash were shaken until clear at ambient temperature. Radioactivity was counted by a liquid scintillation counter (LSC, Packard, Meriden, CT).

^{125}I Administration

A limited time-course kinetic study was conducted with carrier free ^{125}I mixed with cold iodide in physiological saline (33 μg/kg). Naïve adult male rats were dosed intravenously with ^{125}I to aid in the construction of a PBPK model for the early distribution (first two hours after administration) of radiolabeled iodide. Bound and total ^{125}I radioactivity were measured in serum, thyroid and urine. Free ^{125}I radioactivity was determined by subtracting the total ^{125}I radioactivity from bound ^{125}I radioactivity. Free ^{125}I represents the inorganic or free form of ^{125}I.

Bound ^{125}I radioactivity is referred to as bound iodine. Methods for this study are detailed in Yu *et al.* (2000).

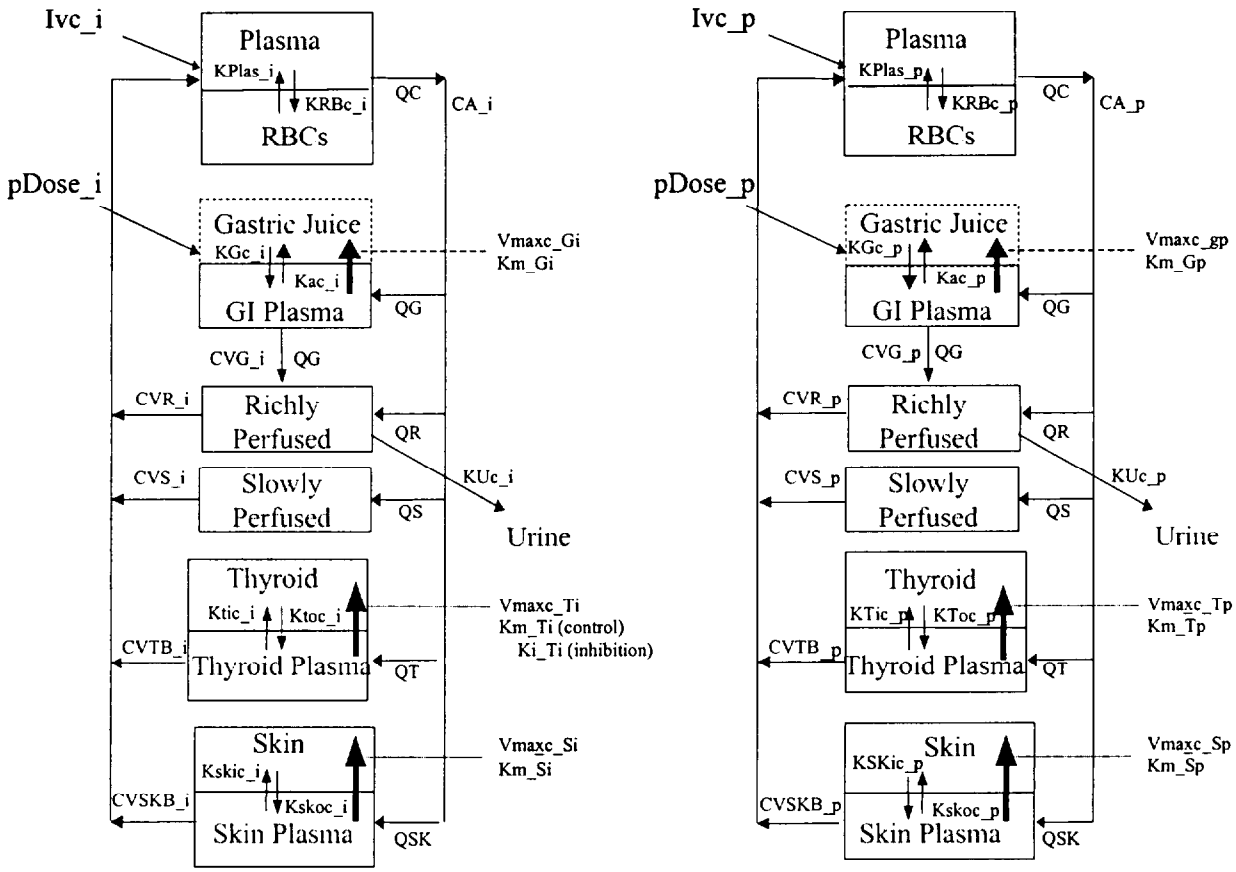
Inhibition of ^{125}I Uptake in the Thyroid

A series of kinetic studies were undertaken to develop a PBPK model that described perchlorate induced inhibition of uptake of ^{125}I in the thyroid gland in adult male rats. Naïve rats were first dosed intravenously in the tail vein with perchlorate (0.01, 0.1, 1.0 and 3.0 mg/kg), then two hours later dosed intravenously in the tail vein with carrier free ^{125}I mixed with cold iodide in physiological saline (33 μg/kg). Rats were killed at selected times (n=6 per time point). Bound iodine, free ^{125}I and perchlorate were measured in serum, thyroid and urine. Methods are provided by Yu *et al.* (2000).

PBPK Model Development and Limitations

The PBPK model for $^{36}ClO_4$ (Fig. 1) consists of lumped compartments (slowly and richly perfused), serum/red blood cell compartment and organs that display active uptake of $^{36}ClO_4$. Organs with organ: serum $^{36}ClO_4$ concentration ratios greater than 1 (skin, thyroid and gastrointestinal content) were assumed to display active uptake kinetic behavior. Only the mass of $^{36}ClO_4$ was accounted for in the gut content compartment and is designated with a dotted line. Loss of $^{36}ClO_4$ from the body occurred by urinary excretion and was described as a first order loss from the richly perfused compartment. The ^{125}I PBPK model (Fig. 1) was developed with fewer data than the perchlorate PBPK model, using only 24-hour urine collections and time course data in serum (free ^{125}I) and thyroid (total ^{125}I as iodine). Therefore, active uptake of iodide into the skin and gastrointestinal tract was not experimentally determined, but was assumed to equal the uptake of $^{36}ClO_4$. Perchlorate probably inhibits uptake of iodide in the gastrointestinal tract and skin in addition to the thyroid gland. Inhibition of iodide uptake by perchlorate was not investigated in the skin and gastrointestinal tract. Thus, perchlorate was not assumed to inhibit uptake of iodide for these compartments.

Figure 1. Schematic of PBPK models for perchlorate (right) and ^{125}I-iodide (left). Competitive inhibition of uptake of ^{125}I by perchlorate is described by a competitive inhibition equation in which the affinity constant for ^{125}I (*Km_i*) is increased (*Ki_i*).



The free iodide partition coefficients for richly and slowly perfused tissues (Table 1) were estimated by averaging muscle: serum and liver: serum ratios obtained from the terminal phase of several time course data sets (Halmi *et al.*, 1956). The perchlorate richly and slowly perfused partition coefficients (Table 1) were estimated by averaging the muscle:serum and liver:serum ratios obtained from the terminal phase of our $^{36}ClO_4$ study. Physiological values (e.g., blood flows, tissue volumes and blood volumes) for the rat model were obtained from the literature (Table 2).

TABLE 1. CHEMICAL SPECIFIC PARAMETERS

Partition Coefficient[a] (unitless)	**Iodide**	**Perchlorate**	**Source**
Slowly perfused / plasma *PS_*	0.21	0.31	Iodide – (Halmi *et al.*, 1956) ClO_4 - $^{36}ClO_4$ study
Rapidly perfused / plasma *PR_*	0.80	0.56	Iodide – (Halmi *et al.*, 1956) ClO_4 - $^{36}ClO_4$ study
***Max Capacity, Vmaxc* (ng/hr/kg)[b]**	**Iodide**	**Perchlorate**	**Source**
Thyroid *Vmaxc_T*	4.0E4	5.0E3	Fitted
Skin *Vmaxc_S*	1.0E8	1.0E8	Fitted, $^{36}ClO_4$
Gut *Vmaxc_G*	5.0E7	5.0E7	Fitted, $^{36}ClO_4$
Affinity Constant, *Km*[b] (ng/L)	**Iodide**	**Perchlorate**	**Source**
Thyroid *Km_T*	3.96E6	3.96E6	Gluzman & Niepomniszcze, 1983
Skin *Km_S*	3.96E6	3.96E6	Gluzman & Niepomniszcze, 1983
Gut *Km_G*	3.96E6	3.96E6	Gluzman & Niepomniszcze, 1983
Inhibition Constant, *Ki*[b] (ng/L)	**1 mg/kg Perchlorate**	**3 mg/kg Perchlorate**	**Source**
Thyroid *Ki_Ti*	1.0E7	1.5E7	Fitted
First Order Rate Constant, *K*[b] (/hr-kg)	**Iodide**	**Perchlorate**	**Source**
Urinary excretion *Kuc*	0.5	0.5	Fitted
Thyroid blood to thyroid *KTic*	0	2	Fitted
Thyroid to thyroid blood *KToc*	0	2	Fitted
Gut content to gut blood *KGc*	100	100	Fitted
Bi-directional - plasma to RBCs *KRBC*	100	80	Fitted
Skin blood to skin *KSKic*	100	100	Fitted $^{36}ClO_4$
Skin to skin blood *KSKoc*	100	100	Fitted $^{36}ClO_4$

Notes: [a] All parameters listed are notated in the model by either an *i* (for iodide) or *p* (for perchlorate) following the parameter name (e.g., *PR_i, PR_p, Vmaxc_Ti, Vmaxc_Tp, etc.*).

[b] Scaled by bodyweight (BW) as follows:

$$V\max_Xy = V\max c_Xy \times BW^{3/4}$$

$$KX_y = KXc_y / BW^{1/4}$$

where: *X = compartment of concern (e.g., G for gut, T for thyroid, etc.)*
y = anion of concern (i for iodide, p for perchlorate)

TABLE 2. PHYSIOLOGICAL PARAMETERS

Parameter	Value	References
Volumes		
Plasma V*plasc* (%BW)	3.7	
RBCs *VRBCc* (%BW)	3.7	
Thyroid *VTc* (%BW)	0.0065	Unpublished data
Thyroid Capillary Blood *VTBc* (%VT)	18.1	18.1% thyroid wt in rat (Brown *et al.*, 1997)
Skin *VSkc* (%BW)	19	Brown *et al.*, 1997
Skin Capillary Blood *VSkBc* (%VSk)	2	Brown *et al.*, 1997
Gut (without contents) *VGc* (%BW)	3.6	Brown *et al.*, 1997
Gut Capillary Blood *VGBc* (%VG)	2.9	Altman and Dittmer, 1971
Slowly Perfused *VSc* (%BW)	53	Lumped compartment
Richly Perfused *VRc* (%BW)	11	Lumped compartment
Blood Flows		
Cardiac Output *QCc* (L/hr/kg)	14	
Thyroid *QTc* (%QC)	1.6	Brown *et al.*, 1997
Gut *QGc* (%QC)	13.6	Brown *et al.*, 1997
Skin *QSKc* (%QC)	5.8	Brown *et al.*, 1997
Slowly Perfused *QS*	16.6	Lumped compartment
Richly Perfused *QR*	62.4	Lumped compartment

The initial approach for model development was to construct a PBPK model for perchlorate using a single dose of $^{36}ClO_4$ (3.3 mg/kg). Urinary excretion of $^{36}ClO_4$ was rapid with about 88 and 95% of the administered dose excreted at 12 and 24 hours, respectively. While in the body, $^{36}ClO_4$ was sequestered into the thyroid, skin and gastrointestinal tract. Perchlorate was assumed to enter the thyroid, skin and gastrointestinal tract by two processes, active uptake (via the NIS) and passive diffusion. The active sequestration from tissue plasma into the tissue was described by Michaelis-Menten kinetics (Table 1) for each tissue. Uptake and clearance of $^{36}ClO_4$ between tissue and tissue plasma by passive diffusion were described as 1st order processes (Fig. 1). The 1st order rate constant values for passive diffusion were obtained by fitting $^{36}ClO_4$ concentrations as it cleared the tissue. The passive diffusion rate constant for uptake of $^{36}ClO_4$ into tissue was assumed to equal the clearance rate constant. The Michaelis-Menten affinity constants (Km) for $^{36}ClO_4$ and ^{125}I were set equal to each other and for each tissue (skin, thyroid and gastrointestinal tract). The experimentally determined *in vitro* Km values for uptake of ^{125}I in the thyroid by the NIS are similar across species (Gluzman and Niepomniszcze, 1983) and across laboratories

(Wolff and Maurey, 1963). Km values for uptake of ^{125}I in non-thyroidal tissues are similar to the thyroid; however, the maximum velocity or capacity differs across tissues containing NIS (Wolff and Maurey, 1961).

An iterative process of visually fitting selected model parameters was used (Table 1). Initially the urinary clearance rate constant was estimated to provide a reasonable description of the plasma $^{36}ClO_4$ profile. Then, transfer of $^{36}ClO_4$ between plasma and red blood cells was described as a first order diffusion process. The red blood cell $^{36}ClO_4$ concentrations paralleled the plasma concentrations. The values for the transfer rate constants were selected only on the ability of the simulations to reproduce the red blood cell and plasma time course data. Uptake of $^{36}ClO_4$ into thyroid, skin and gastrointestinal content were described by fixing the value for Km (for each tissue) and adjusting the Vmaxc for each tissue until a fit was achieved between simulation and observation. Passive diffusion (uptake and clearance) constant values were obtained by fitting the $^{36}ClO_4$ clearance portion of the kinetic curve for each tissue. After this procedure was completed, the urinary excretion rate constant was readjusted slightly to provide agreement between simulation and observation and then the above process was carried out again with the thyroid, skin and gastrointestinal content.

The $^{36}ClO_4$ PBPK model served as the template for development of an ^{125}I (with carrier) PBPK model. Only serum and thyroid time course data were collected from a single dose of ^{125}I mixed with cold iodide (33 μg/kg). The PBPK model for ^{125}I with carrier predicts free ^{125}I iodide in serum and total ^{125}I iodine (free and bound) in thyroid. A 24-hour urine sample was taken to estimate the amount of ^{125}I iodide excreted in urine over a 24-hour period. Model parameter values for the urinary excretion rate constant (Kuc_i) and for uptake of iodide into the thyroid (Vmaxc_Ti) were estimated by fitting. The bi-directional diffusion transfer constant for serum and red blood cells was set to 100 /hr. ^{125}I (with carrier) was assumed not to leave the thyroid over the two hour simulation period. ^{125}I radioactivity increased in the thyroid for several hours after dosing. Therefore, the rate constants for diffusion of ^{125}I (with carrier) between thyroid plasma and thyroid were set equal to zero.

RESULTS

The PBPK physiologic and chemical specific model parameters for perchlorate and ^{125}I are found in Tables 1 and 2. Figures 2 A through G provide observed versus model-predicted concentrations or amounts of $^{36}ClO_4$ in tissues. Clearance of $^{36}ClO_4$ in plasma and red blood cells was slightly faster than predicted by the model (Fig. 2A and B). Active uptake of $^{36}ClO_4$ into skin (Fig. 2C) was described using a Vmaxc_Sp value of 1E+8 ng/hr/kg and a Km_Sp set to 3.96E+6 ng/L. Clearance of $^{36}ClO_4$ from skin was slower than clearance from plasma and was described with a diffusion constant (KSKoc) of 100 /hr. Active uptake of $^{36}ClO_4$ in the thyroid was adequately described using a Vmaxc_Tp value of 5,000 ng/hr/kg and a Km_Tp value set to 3.96E+6 ng/L. Clearance of $^{36}ClO_4$ in the thyroid was also slower than plasma and was described using a diffusion constant (KToc) of 2 /hr. Differences in the maximum velocity and the diffusion rate constant values for the skin and thyroid are due to large differences in the volume of the organs. Skin is about 55 g and the thyroid gland is about 15 to 20 mg for a 290 g Sprague-Dawley male rat. Clearance of $^{36}ClO_4$ from muscle was similar to plasma (Fig. 2E).

Figure 2. Male rats were administered ^{36}Cl-perchlorate *iv*. Continuous line is model simulation and vertical line is observed mean concentration (ng/L or ng/kg) or amount (ng) ± standard deviation (SD) in: A) serum, B) red blood cells, C) skin, D) thyroid, E) slowly perfused tissue (muscle), F) gastrointestinal content and G) urine.

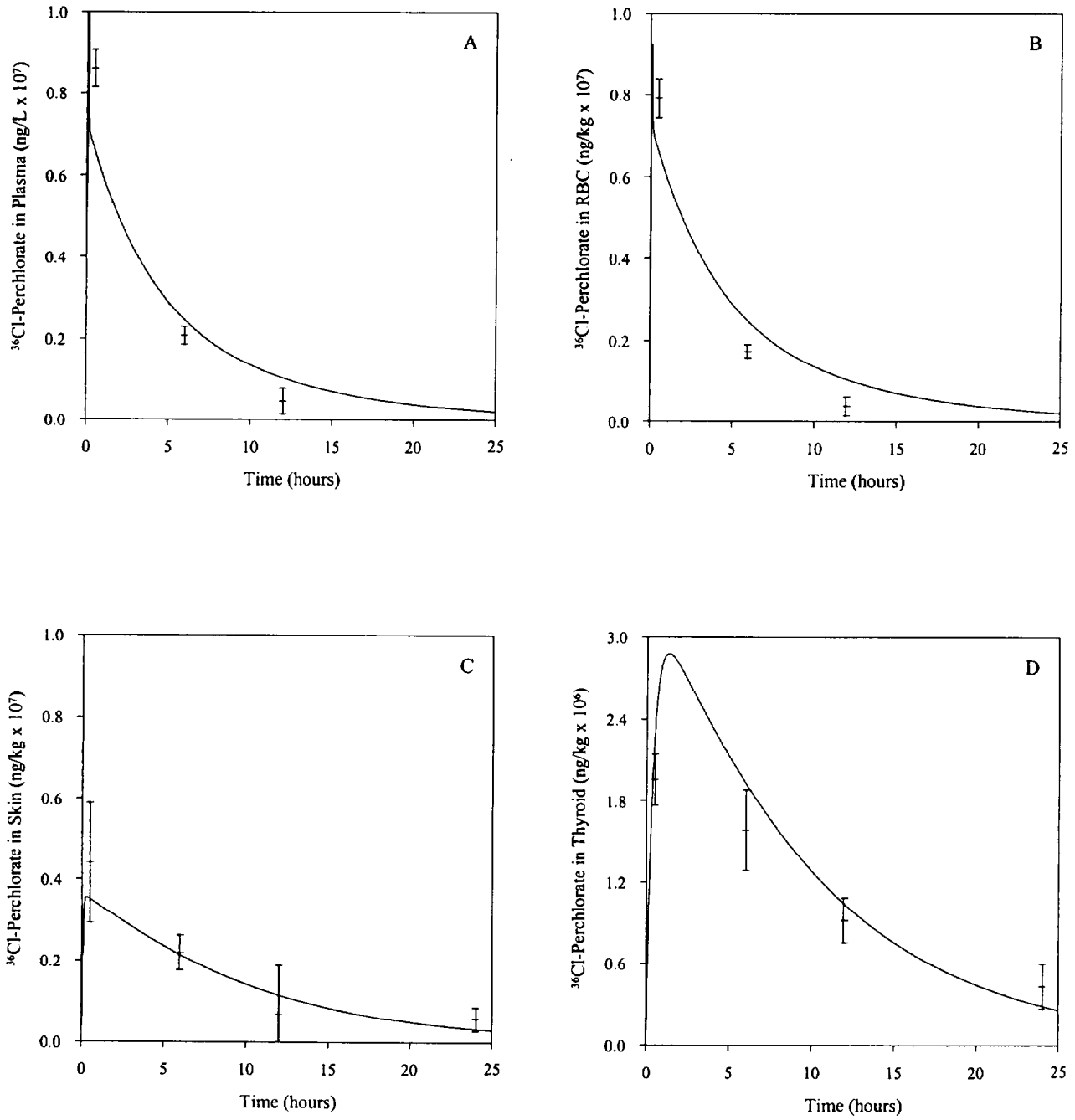
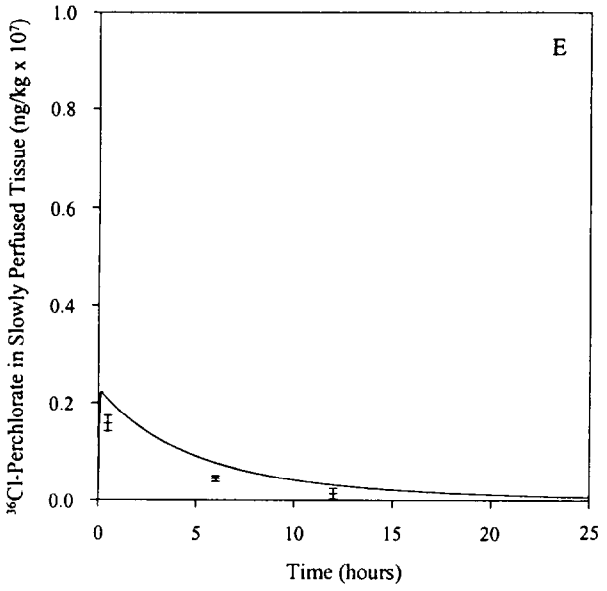
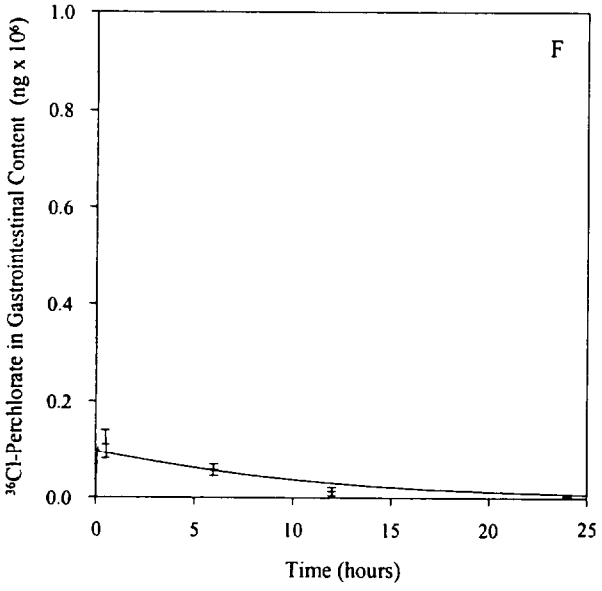
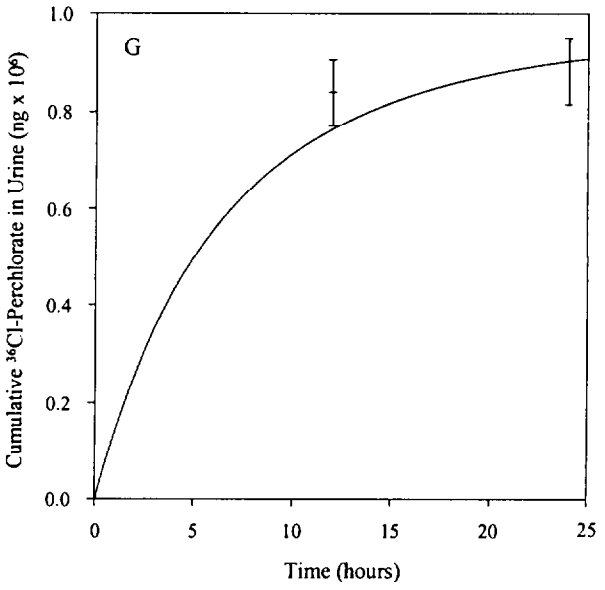


Figure 2. (continued)







The gastrointestinal tract sequestered much of the $^{36}ClO_4$ into the GI content. Very little $^{36}ClO_4$ was found in feces. Therefore, $^{36}ClO_4$ is reabsorbed into the blood supply. Figure 2F depicts the amount of $^{36}ClO_4$ in the gastrointestinal tract content. The active uptake of $^{36}ClO_4$ was described using a Vmaxc_Gp value of 5E+7 ng/hr/kg and a Km_Gp value of 3.96E+6 ng/L. Secretion of $^{36}ClO_4$ into systemic circulation from the GI tract content was described with a diffusion rate constant (Kgc_p) value of 100 /hr. Urinary excretion of $^{36}ClO_4$ occurred quickly and was described with a 1st order rate constant (Kuc_p) value of 0.5 /hr (Fig. 2G). The calibrated model for *iv* administration of 3.3 /g/kg of $^{36}ClO_4$ was successful in simulating several tissue concentration time course data sets.

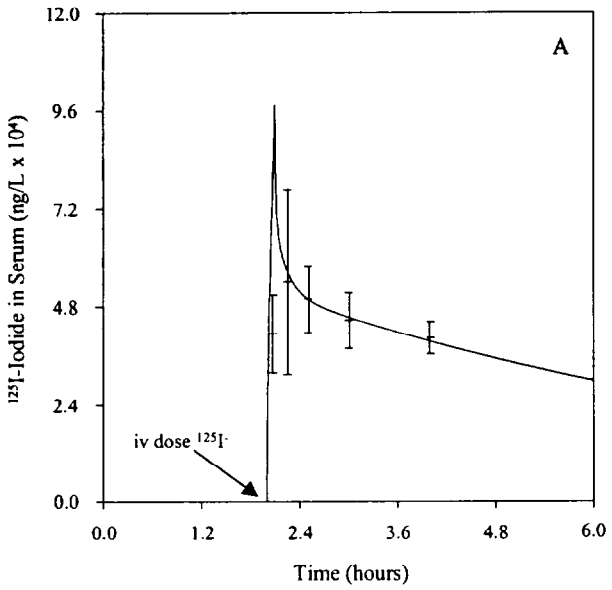
Figure 3A through C depicts model simulation versus observation after an intravenous tail vein dose of 33 μg/kg of ^{125}I. The urinary excretion rate constant Kuc_i was set to 0.5 /hr, which is the same value used for urinary excretion of $^{36}ClO_4$ (Table 1). This provided a good description of clearance of free ^{125}I in serum (Fig. 3A) over a 2 hour period and prediction of the 24-hour cumulative amount of free ^{125}I excreted in urine (Fig. 3C). The active uptake of ^{125}I into the thyroid over a 2 hour period was described with a Vmaxc_Ti value of 40,000 /hr (Fig. 3B). Passive diffusion between the thyroid blood and thyroid was set to zero. All other model parameter values were set equal to values used for $^{36}ClO_4$, with the exception of the transfer rate constants for serum and red blood cells (Table 2).

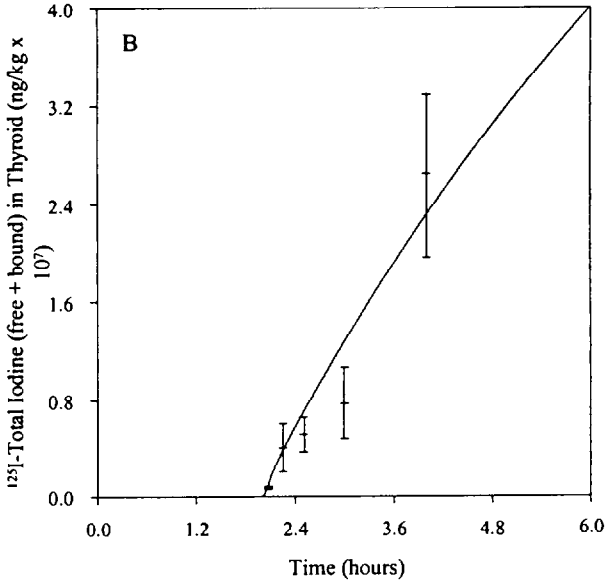
To evaluate the effects of perchlorate on uptake of ^{125}I in the thyroid in the naïve rat, rats were dosed intravenously with perchlorate then challenged two hours later with an intravenous administration of ^{125}I (with carrier). Rats were dosed with 0.01, 0.1, 1.0 and 3.0 mg/kg of perchlorate. Figures 4A through D depict the observed and predicted serum concentrations of perchlorate after *iv* administration of perchlorate. The observed concentrations of perchlorate in serum were greater than predicted by the model for the two lower doses (Fig. 4A and B). The concentrations of perchlorate in the serum for the higher doses (Fig. 4C and D) were slightly lower than predictions. The reason for the discrepancy with the lower doses is unknown. Possible factors influencing the kinetic behavior of perchlorate at low doses in serum are protein binding, red blood cell binding and reduced transfer of perchlorate from blood to tissues that actively sequester perchlorate.

Figure 5A through D depicts the uptake of perchlorate into thyroid after *iv* administration. For the two lower doses (Fig. 5A and B) the uptake of perchlorate was under-predicted. However, the serum levels of perchlorate were also higher than predicted by the model for these doses (Fig. 4A and B). The uptake of perchlorate would be described satisfactorily if the perchlorate serum concentrations were adequately predicted. The uptake of perchlorate in the thyroid for the higher doses (Fig. 5C and D) were slightly under-predicted and clearance from the thyroid at 24 hours was over-predicted. This suggests that the uptake and clearance kinetics of perchlorate in the thyroid may be more complex than anticipated.

Figure 6 compares model predicted and observed urinary excretion of the cumulative amount of perchlorate in urine. A 1st order urinary excretion rate constant value of 0.5/hr was used for all dose groups. Good agreement was obtained between model predicted and observed amounts of perchlorate excreted in urine.

Figure 3. Male rats were intravenously dosed with 33 μg/kg ^{125}I-iodide (with carrier). Abscissa depicts dosing schedule for perchlorate inhibition studies (Experiment 3). No perchlorate was administered in this case.





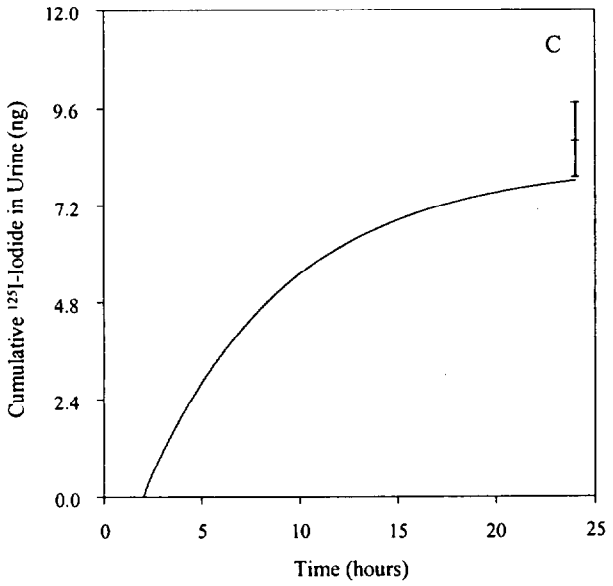


Figure 4. Model predicted and observed concentrations of perchlorate in serum of male rats intravenously administered perchlorate. Solid continuous line represents simulation and vertical lines represent observed mean values ± SD for: A) 0.01 mg/kg perchlorate, B) 0.1 mg/kg perchlorate, C) 1.0 mg/kg perchlorate and D) 3.0 mg/kg perchlorate.

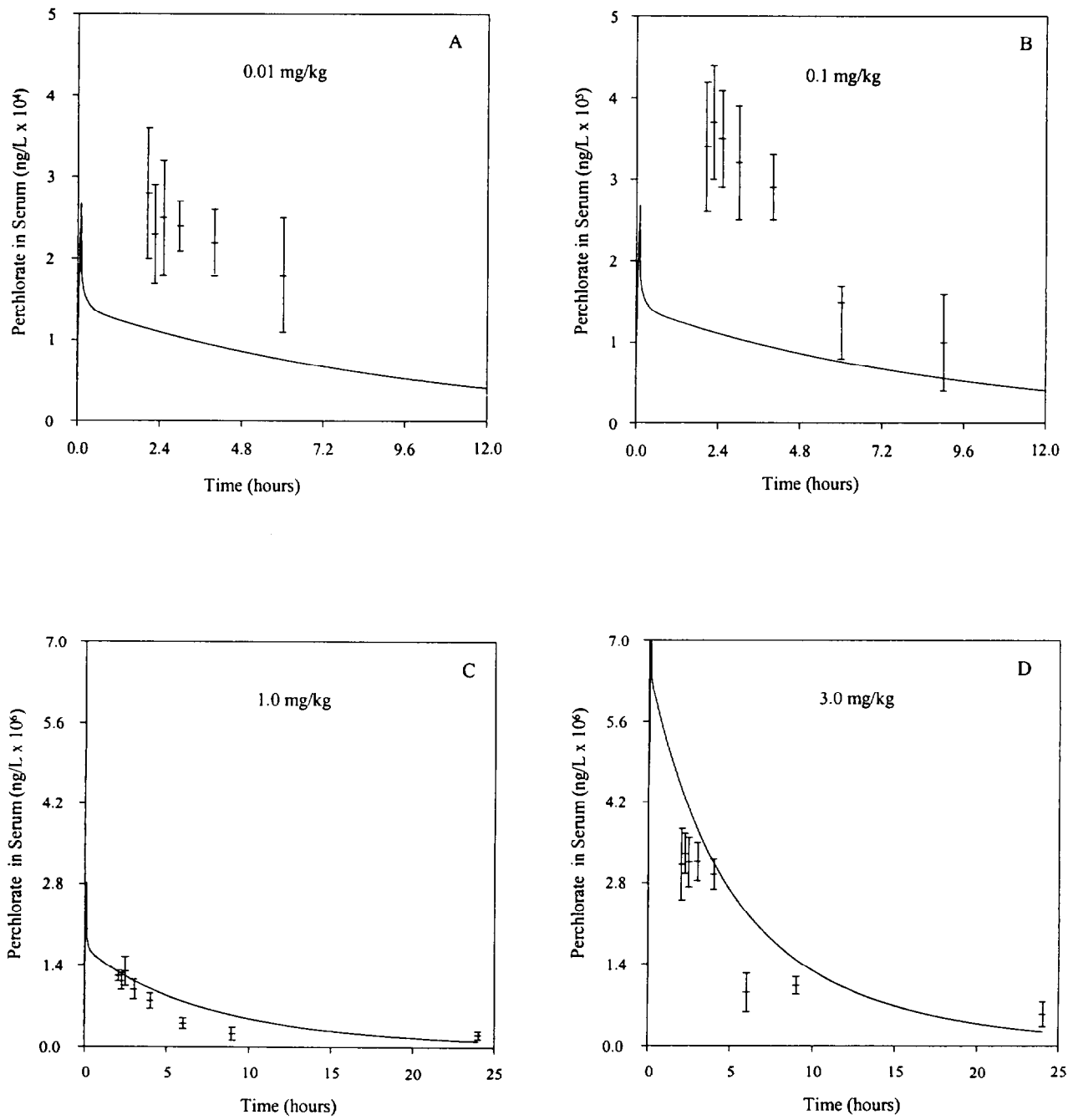


Figure 5. Model predicted and observed concentrations of perchlorate in thyroid of male rats intravenously administered perchlorate. Solid continuous line represents simulation and vertical lines represent observed mean values ± SD for: A) 0.01 mg/kg perchlorate, B) 0.1 mg/kg perchlorate, C) 1.0 mg/kg perchlorate and D) 3.0 mg/kg perchlorate.

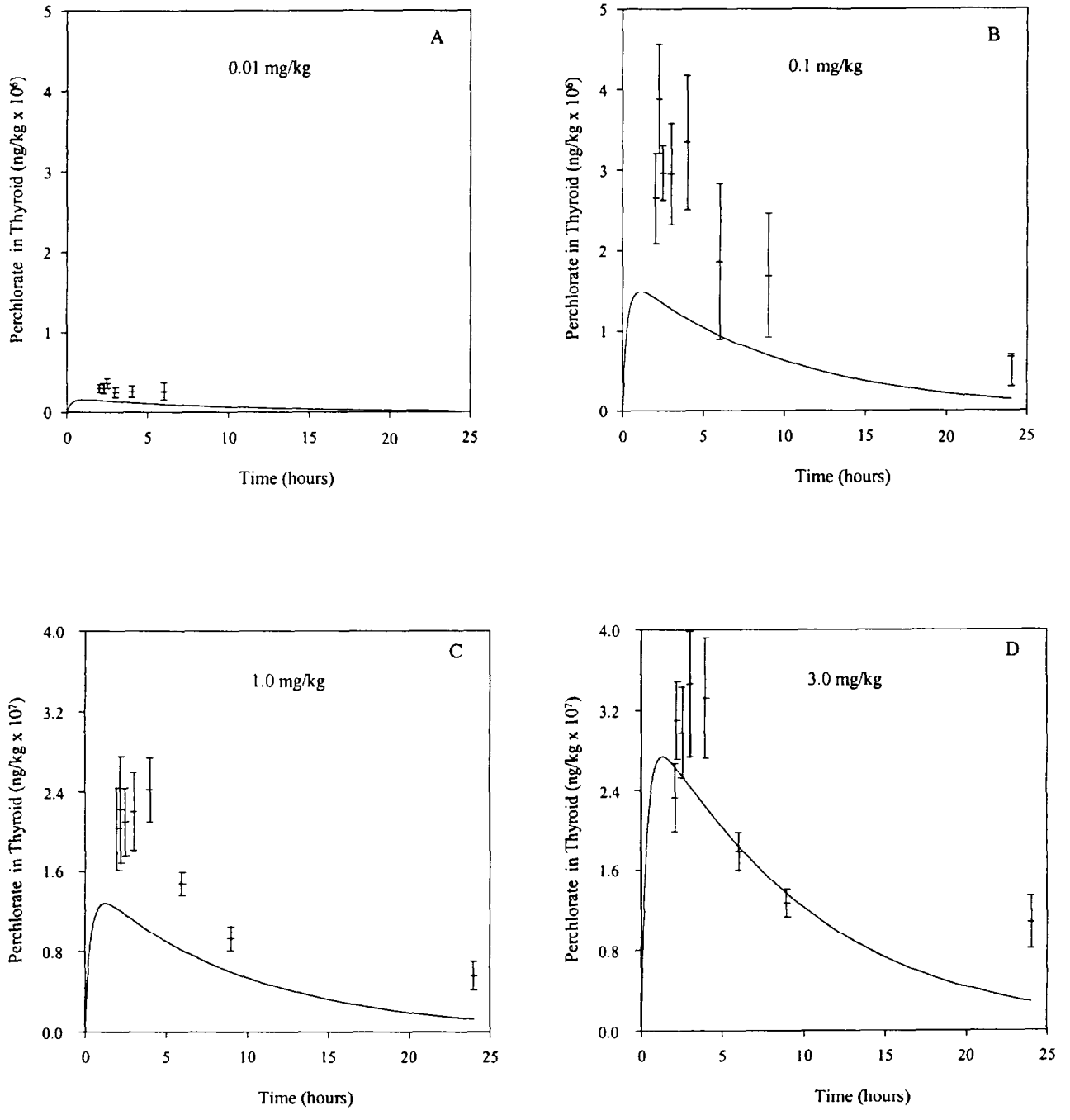
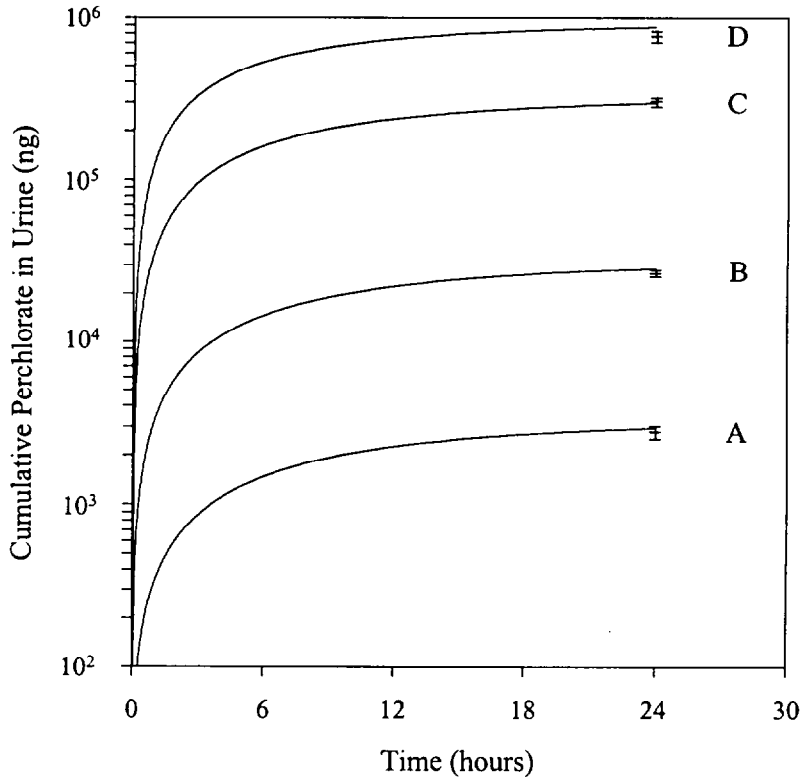


Figure 6. Model predicted and observed cumulative amounts of perchlorate in urine of male rats intravenously administered perchlorate. Solid continuous lines represent simulations and vertical lines represent observed mean values ± SD for: A) 0.01 mg/kg perchlorate, B) 0.1 mg/kg perchlorate, C) 1.0 mg/kg perchlorate and D) 3.0 mg/kg perchlorate.



Figures 7A through F depict time course data for ^{125}I kinetics in serum and urine from rats dosed with 3.0 mg/kg of perchlorate and uptake of ^{125}I in the thyroid of rats dosed with 0.01 to 3.0 mg/kg of perchlorate. The serum ^{125}I kinetic profile in serum (Fig. 7A) and urine (Fig. 7B) was not altered by administration of 0.01 to 3.0 mg/kg perchlorate. The model simulation suggested that slightly more ^{125}I would be excreted in urine 22 hours after dosing in rats dosed with 3.0 mg/kg of perchlorate. The uptake of ^{125}I in the thyroid for all dose groups of perchlorate is shown in Figure 7C. The 1.0 and 3.0 mg/kg perchlorate dose groups had pronounced effects on the uptake of ^{125}I in the thyroid. Simulations predicting the inhibition of uptake of ^{125}I for the 1.0 and 3.0 mg/kg dose groups were accomplished by increasing the value of Ki_Ti from 3.96E+6 to 1.0 E+7 and 1.5 E+7 ng/L, respectively. Figures 7D through G depict ^{125}I uptake for each perchlorate dose group over a two hour period. Good agreement was obtained between observation and model prediction across dose groups.

SUMMARY AND DISCUSSION

Physiologically based pharmacokinetic models for ^{125}I and perchlorate describes the competitive inhibition of uptake of ^{125}I in the thyroid by perchlorate. Although urinary excretion of both anions (^{125}I and $^{36}ClO_4$) accounts for the majority of the dose, both anions are actively sequestered into thyroid and non-thyroid tissues. Active uptake of perchlorate into tissues appears to slow systemic clearance of perchlorate and ^{125}I. This PBPK model only evaluated the effect of perchlorate on the uptake of ^{125}I on the thyroid. Active uptake of ^{125}I into the skin and gastrointestinal content is probably altered by perchlorate. However, the serum time-course data of ^{125}I over a two hour period after *iv* administration were similar in the control (Fig. 3A) and 3.0 mg/kg perchlorate dose group (Fig. 7A). This suggests that, for the dose range of perchlorate, inhibition of ^{125}I uptake from serum into tissues had little impact on the serum ^{125}I time course profiles (Yu *et al.*, 2000).

The mean percent inhibition of uptake of ^{125}I into thyroid at 2 hours after dosing was 13, 24, 70 and 88% for the 0.01, 0.1, 1.0 and 3.0 mg/kg dose groups, respectively, compared to control. The 1.0 and 3.0 mg/kg perchlorate doses exhibited the most pronounced inhibitory effects that persisted for 22 hours with ^{125}I-total iodine concentrations in the thyroid 35 and 63% below control values (data not shown). The thyroid gland is capable of overcoming the inhibitory effects of perchlorate (Yu *et al.*, 2000) for perchlorate doses that do not cause severe inhibition.

The PBPK model was not successful over the entire dose range (0.01 to 3.0 mg/kg) in describing the pharmacokinetics of perchlorate in naïve rats dosed with a single dose of perchlorate. Further investigation is needed to examine the reason for the apparent elevated serum levels of perchlorate in the low dose groups and the inability to simulate the uptake and clearance of perchlorate in the thyroid. Further research is needed to determine the nature of ^{125}I sequestration in skin and the gastrointestinal tract of the adult male rat and the impact perchlorate has on iodide uptake into these tissues.

Figure 7. Iodide uptake inhibition in male rats intravenously administered perchlorate, followed by 33 μg/kg ^{125}I-iodide (with carrier) challenge two hours later. Continuous line is model simulation and vertical lines are observed mean values ± SD for: A) concentration of ^{125}I in serum following 3 mg/kg perchlorate, B) cumulative amount of ^{125}I in urine following 3 mg/kg perchlorate, C) concentrations of total ^{125}I in thyroid after 0, 0.01, 0.1, 1.0 and 3.0 mg/kg perchlorate, D) concentration of total ^{125}I in thyroid after 0.01 mg/kg perchlorate, E) concentration of total ^{125}I in thyroid after 0.1 mg/kg perchlorate, F) concentration of total ^{125}I in thyroid after 1.0 mg/kg perchlorate and G) concentration of total ^{125}I in thyroid after 3 mg/kg perchlorate.

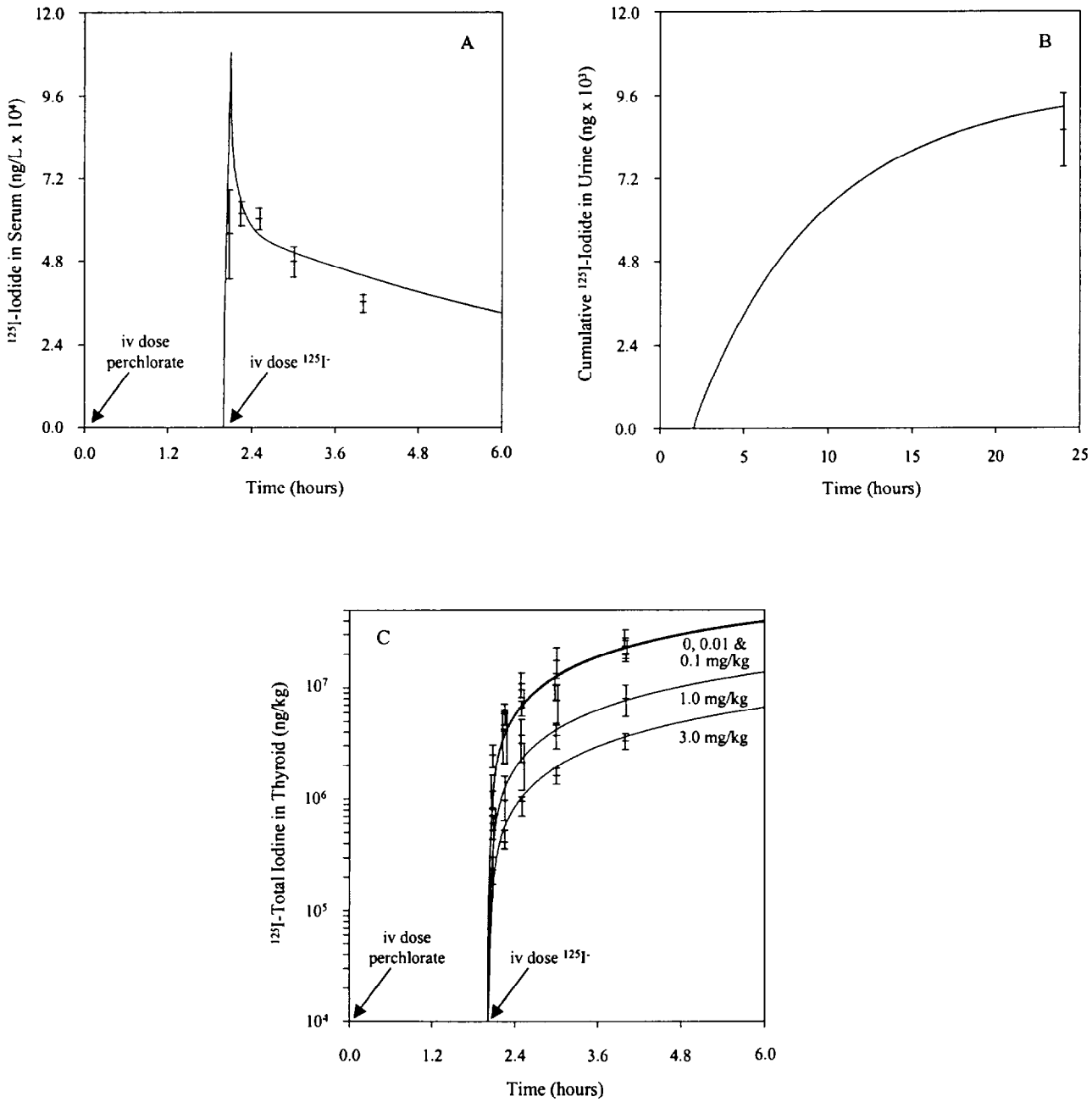
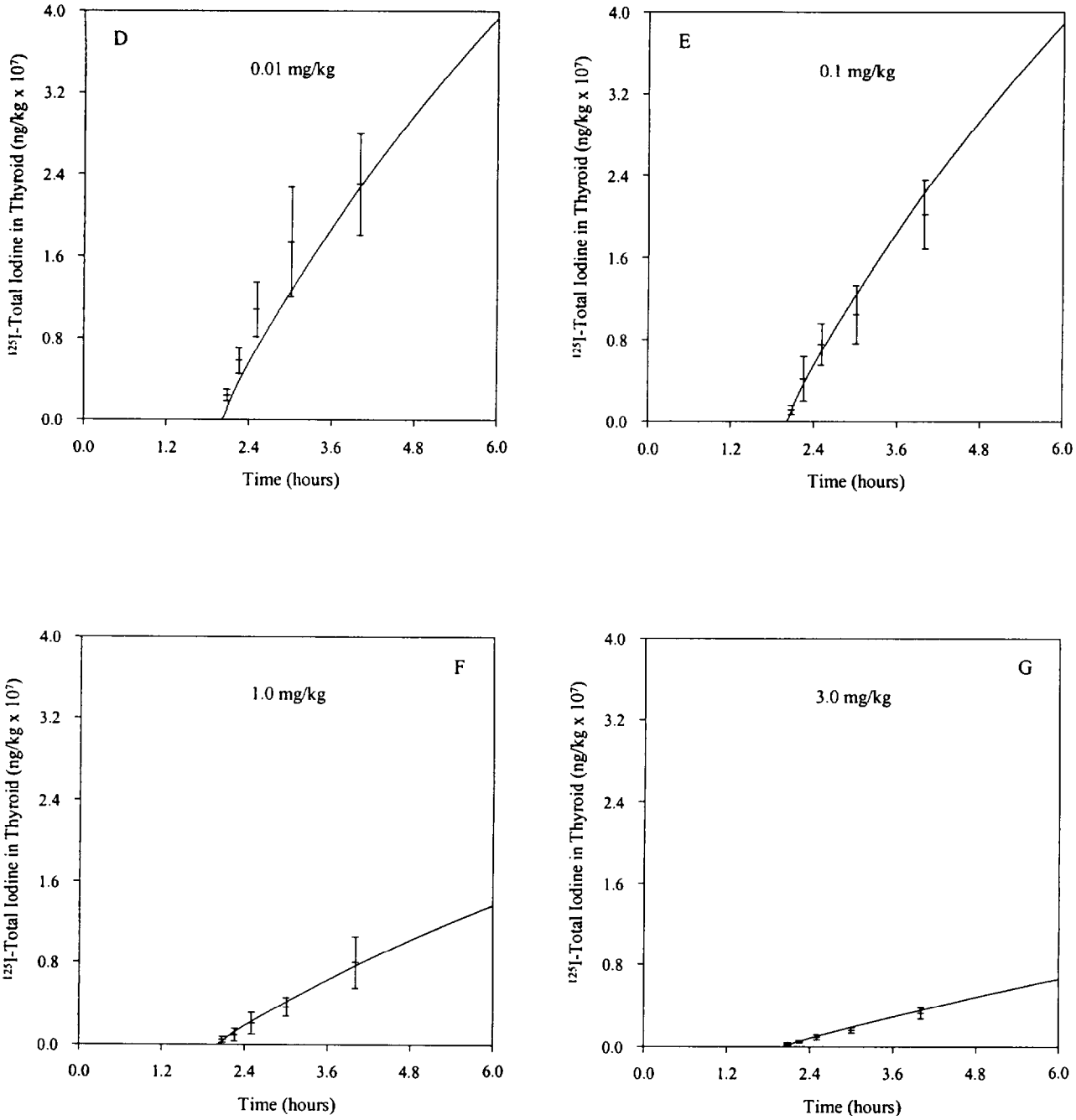


Figure 7. (continued)



These studies will be the basic for development of a biologically based physiological (BBP) model for thyroid hormone homeostasis. This PBPK model only simulated the early distribution (first two hours) of ^{125}I in the body because loss of ^{125}I to thyroid hormone synthesis and appearance of ^{125}I from metabolism of thyroid hormones was not accounted for in the model. The error introduced into the model by not accounting for thyroid hormone formation and metabolism of thyroid hormones (including inactive and incomplete forms of thyroid hormones) was thought to have a very minimal influence on the model predictions of free ^{125}I in serum over a brief period of time. The BBP model will account for endogenous free iodide, TSH and T_4 in addition to perchlorate and the feedback loops that govern thyroid hormone homeostasis.

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