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A Physiologically Based Pharmacokinetic Model for Arsenic Exposure

I. Development in Hamsters and Rabbits

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A physiologically based pharmacokinetic model for exposure to inorganic arsenic in hamsters and rabbits has been developed. The model in its present state simulates three routes of exposure to inorganic arsenic: oral intake, intravenous injection, and intratracheal instillation. It describes the tissue concentrations and the urinary and fecal excretions of the four arsenic metabolites: inorganic As(III) and As(V), methylarsonic acid, and dimethylarsinic acid. The model consists of five tissue compartments, chosen according to arsenic affinities: liver, kidneys, lungs, skin, and others. The model is based on physiological parameters, which were scaled according to body weight. When physiological parameters were not available, the data for the model were obtained by fitting (tissue affinity, absorption rate, and metabolic rate constants). The excretions of the arsenic metabolites in urine and feces are well simulated with the model for both species. Further validation of the arsenic metabolite concentrations in the tissues and *in vitro* measurements of the tissue affinity constants are discussed.

Occupational exposure to arsenic occurs mainly by inhalation of particulate containing arsenic in the smelting of nonferrous ores, in the production and use of arsenic-containing pesticides, in the production of glass, and in electronic industries producing arsenides of gallium or indium or high-speed III–V semiconductors out of these compounds. In the general population, drinking water is a major source of inorganic arsenic. Elevated concentrations in the drinking water may be due to leaching from arsenic-rich bedrock or to pollution. Inorganic arsenic is known to cause skin lesions, including skin cancer, and cancer of internal organs following ingestion (WHO, 1981; Bates *et al.,* 1993), and lung cancer following inhalation (IARC, 1980).

Most mammals are able to methylate inorganic arsenic to methylarsonic acid (MMA) and dimethylarsinic acid (DMA) (for review, see Vahter and Marafante, 1988; Vahter, 1994). All the different steps in the methylation of arsenic are not known, but it seems likely that it takes place mainly in the liver by sequential transfer of methyl groups from *S*-adenosylmethionine to arsenic in its trivalent form (Marafante and Vahter, 1984; Buchet and Lauwerys, 1985). Absorbed arsenate (AsV) is to a large extent reduced in the blood to AsIII (Vahter and Envall, 1983; Marafante *et al.,* 1985; Vahter and Marafante, 1985; Yamauchi and Yamamura, 1979). The methylation of inorganic arsenic in mammals is generally considered a detoxification mechanism. Compared to inorganic arsenic, the methylated metabolites are much less toxic (Squibb and Fowler, 1983). In most mammals they are less reactive with tissue components and rapidly excreted in the urine (Buchet *et al.,* 1981; Vahter and Marafante, 1983; Vahter *et al.,* 1984); Marafante *et al.,* 1987).

There are major species differences in the urinary excretion of the methylated arsenic metabolites following exposure to inorganic arsenic, indicating significant differences in the rate of methylation of inorganic arsenic (Vahter and Marafante, 1988; Vahter, 1994). Only human subjects excrete significant amounts of MMA, with 10–20% of the urinary arsenic being in the form of MMA (Buchet *et al.,* 1981; Tam *et al.,* 1979; Vahter, 1986; Johnson and Farmer, 1991). Mice and dogs methylate arsenic very efficiently and a major part of the urinary arsenic is in the form of DMA (Vahter, 1981; Charbonneau *et al.,* 1979; Hollins *et al.,* 1979). Rats also have an efficient methylation of inorganic arsenic, but most of the produced DMA is accumulated in the erythrocytes, resulting in a slow urinary excretion of arsenic (Vahter, 1981; Vahter *et al.,* 1984; Odanaka *et al.,* 1980; Lerman and Clarkson, 1983). Furthermore, compared to other species, the rat has a more pronounced biliary excretion of arsenic (Klaassen, 1974). The marmoset monkey (Vahter *et al.,* 1982); Vahter and Marafante, 1985) and the chimpanzee (Vahter *et al.,* 1995) are the only species which have been shown to be completely unable to methylate inor-

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ganic arsenic. The rabbits and the hamsters seem to be the species most similar to humans with regard to the methylation of arsenic, although they excrete more DMA and less MMA than humans do (Vahter and Marafante, 1988; Vahter, 1994). However, the gastrointestinal absorption of both inorganic and the methylated metabolites is somewhat lower in the hamster than in most other species (Marafante *et al.*, 1987; Marafante and Vahter, 1987; Yamauchi and Yamamura, 1984, 1985).

Physiologically based pharmacokinetic (PB-PK) models are now used more often in risk assessment, since they allow the introduction of biological concepts in extrapolations. They are often used in dose extrapolations and in comparisons between different animal species. In the present work, a PB-PK model is proposed to understand differences in the metabolism between species. Such a model would allow, with further developments, an extrapolation to humans and an integration of our knowledge about arsenic pharmacokinetics, in order to compare As(V) pulmonary exposure either with As(III) pulmonary exposure or with As(V) oral exposure in humans.

This work is a first attempt to interpret pharmacokinetic data on arsenic in animals in a common structure. This PB-PK model is developed for oral and intratracheal exposure in rabbits and hamsters. Its extrapolation and application to humans will be presented in a subsequent paper (Mann *et al.*, 1996).

METHOD

Description of the physiologically based pharmacokinetic model. The model consists of five tissue compartments, chosen according to arsenic affinities: liver, kidneys, lungs, skin, and others containing the remaining tissues, including bones and muscles. Blood makes up one compartment, with two subcompartments at equilibrium: plasma and red blood cells. Only the plasma subcompartment is considered for distribution of arsenic to the tissues (Fig. 1).

Three routes of exposure are considered in the model (Fig. 1): peroral administration (po), intratracheal instillation (i.t.), and intravenous injection (iv). The first route (po) involves absorption in the gastrointestinal tract (GI tract), which is considered a transit compartment (nontissue). The second route (i.t.) involves three transit compartments (nontissue): nasopharynx (NP), tracheobronchial (TB), and pulmonary (P) (ACGIH, 1993). The absorption from these compartments into the liver from GI tract and into the plasma from the lungs is described using first-order kinetics. To stimulate intratracheal instillation, arsenic is considered to be deposited only in the P and TB regions in equal amounts. The NP region is not used in the present discussion, but will be discussed in a subsequent paper on inhalation exposure of humans (Mann *et al.*, 1996). Following po administration,

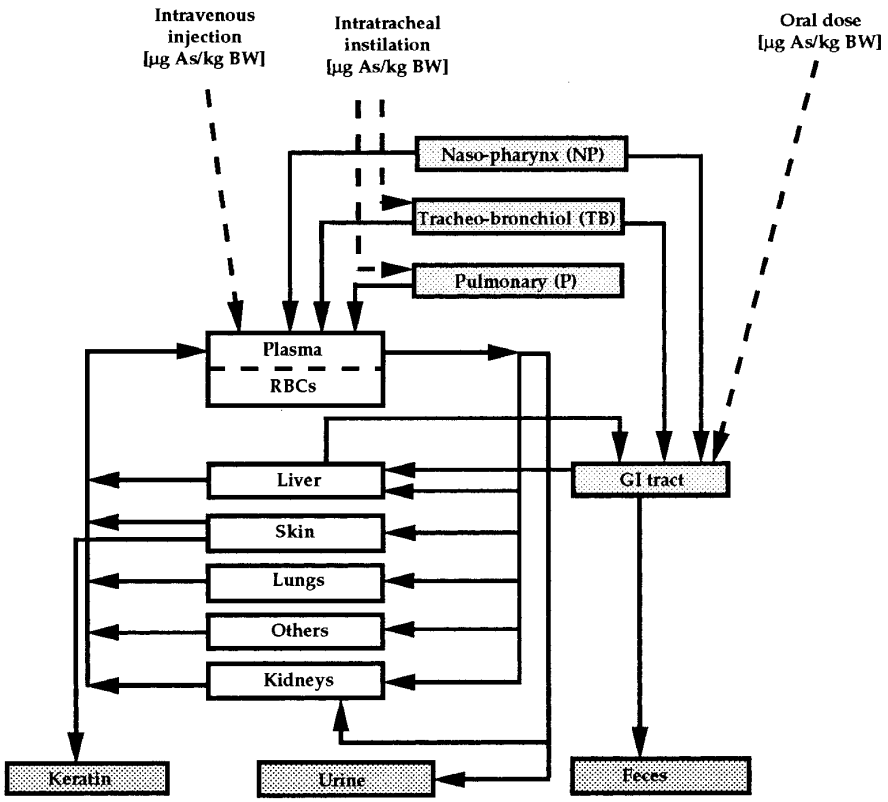

FIG. 1. Schematic presentation of the PB-PK model showing the exposure routes, the tissues distribution, and the excretion routes for one arsenic metabolite. This diagram is repeated four times, one for each arsenic metabolite.

TABLE 1
Capillary Properties Used for Model Development (Karnasovsky, 1970)

Organ	Capillary type	Pore size (Å)	Total pore area (%)	Capillary thickness (μm)
Lungs	Continuous	40	0.1	0.5
Liver	Discontinuous	200	0.02	1
Kidneys	Fenestrated	400	0.06	1
Skin	Continuous	40	0.1	0.5
GI tract	Fenestrated	400	0.06	1
Others	Continuous	40	0.1	0.5

arsenic is diluted in the contents of the stomach and then made available for absorption in the small intestine. For intravenous injection, the dose is introduced as a single bolus into the plasma compartment.

The distribution of arsenic to the tissues depends on their blood perfusion, the permeability of the capillary membranes, and the affinity of the tissues for the arsenic metabolites. For the PB-PK model, blood flows, blood volumes, tissue volumes, capillary surface areas, and glomerular filtration rate represent physiological reference data. These parameters are scaled according to body weight in order to make possible simulation of different animal species (Lindstedt, 1992; Fiserova-Bergerova, 1983).

Arsenic distribution to the tissues is described using a diffusion-limited model. For membrane transfer, nonionized compounds diffuse freely through the capillary membranes, while ionized compounds diffuse only through the pores of the membranes (Berner and Cooper, 1985). Transfer across the capillary membranes is considered to be due to diffusion through the pores for As(V), MMA, and DMA (which are ionized mostly at physiological pH) and to diffusion across the entire capillary area for As(III) (nonionic at physiological pH). Both transfer mechanisms are dependent on the properties of the capillaries (permeability $P_{i,j}$ and membrane type), blood flow, tissue volume, tissue affinity, and arsenic compound property (diffusion coefficient). The capillary properties of the tissues are presented in Table 1. The capillary surface area for each tissue was obtained according to the total capillary surface area (scaled to body weight) and the ratio between the cardiac output and the blood flow for the corresponding tissue. No consideration of active transport was made at this stage of the development. The transfer of As(V) may occur via the phosphate uptake mechanism to the kidneys, but not to liver (Ginsburg, 1965; Lermann and Clarkson, 1983). The consideration of diffusion only for distribution into tissues can underestimate rates of transfer. Tests with higher rates did not produce significant changes in the results obtained.

Affinities of arsenic metabolites (j) for the different tissues (i) are considered to be linear and independent of the animal species. They are described by affinity constants, which are obtained by fitting the model to experimental data.

In the present state the transfer from the plasma to the tissues is dependent on the permeability of the capillaries $P_{i,j}$ and the tissue affinity constants $K_{i,j}$. These two parameters are linked in the model and any underestimation of membrane transport will be reflected in the affinity constants. Therefore only their combination is estimated with certainty.

The biotransformation of arsenic in the body consists of an oxidation/reduction and two methylation reactions. The oxidation/reduction of inorganic arsenic takes place mainly in the plasma (Marafante *et al.*, 1985). It is described in the PB-PK model by first-order mechanisms, where k_{red} and k_{ox} are the first-order rate constants. According to data for As(V) excretion in the dog (Ginsburg, 1965), reduction of As(V) was also included in the kidneys (first-order rate constant k_{redkid}). Based on several studies showing that As(III) methylation takes mainly place in the liver cytosol with enzymatic catalysis (Vahter and Marafante, 1988; Buchet and Lauwerys, 1987, 1988), the model describes the two methylation steps as occurring in the liver by Michaelis–Menten (M-M) reactions only. Methylation of As(III) to MMA is described by $K_{M_{MMA}}$ and $V_{M_{MMA}}$, respectively (M-M constant and maximum rate constant). The second methylation step, methylation of MMA to DMA, is represented by $K_{M_{DMA}}$ and $V_{M_{DMA}}$, respectively. At this stage of the model, no inhibition of the second methylation is considered (Hopenhayn-Rich *et al.*, 1993). All metabolic rate constants are obtained by fitting the model to experimental data. As there are significant species differences in the metabolism of arsenic, the metabolic rate constants are fitted separately for each animal species. In the absence of convincing data for the opposite, the metabolic rate constants are assumed to be the same for single and repeated absorption (no induction of enzymatic systems).

Elimination of arsenic occurs mainly via urine and to some extent also via feces and desquamation of the skin (and other epithelial cells, where arsenic is bound, mainly to keratine). The latter is simulated in the PB-PK model with a keratin elimination compartment. Renal excretion is quantified using experimental data for dogs exposed to As(V) (Ginsburg, 1965). When arsenate was injected, the urinary excretion of As(V) was 75% of the glomerular filtration, with 25% being reabsorbed. The urinary excretion of As(III) was slightly higher than the GFR: there was a filtration equal to the GFR and a secretion equivalent to 20% of the reabsorbed As(V). Urinary elimination rates of MMA and DMA are assumed to be equal to the GFR. The GFR is scaled according to body weight (Fiserova-Bergerova, 1983). In order to make the fecal excretion rate specific for the animal species, the elimination rate of the As metabolites in feces is obtained by using the rate of the food passage in the small intestine (first-order mechanism). This food passage is related to the length of the small intestine, which is obtained by scaling to bodyweight (Karasov *et al.*, 1986; Ruckebusch *et al.*, 1981). Arsenic elimination via keratin represents the binding of As(III) to keratin in hair, nails, and skin cells. This is simulated with an irreversible first-order kinetic for As(III) from the skin compartment to keratin.

TABLE 2
Scaling Parameters Used in the Model

Physiological parameter	a	b	Reference
Blood volume (ml)	71.5	1.01	Lindsted (1992)
Organ weights (g)			
Liver	38.7	0.911	Lindsted (1992)
Kidneys	8.46	0.851	Lindsted (1992)
Lungs	9.09	0.98	Lindsted (1992)
Skin	136	0.9	Lindsted (1992)
Lumen volume (ml)			
Stomache	4.524	0.966	Karasov *et al.* (1986); ICPR (1992)
Small intestine	5.878	0.989	Karasov *et al.* (1986); ICPR (1992)
Blood flows (ml/sec)			
Cardiac output	3.61	0.752	Lindsted (1992)
Liver, hepatic	0.142	0.85	Lindsted (1992)
Liver splanchic	0.608	0.785	Lindsted (1992)
Kidneys	0.654	0.75	Lindsted (1992)
Lungs	0.075	0.837	Lnidsted (1992)
Skin	0.248	0.752	Lindsted (1992)
Clearance (ml/sec)			
GFR	0.065	0.79	Lindsted (1992)
Small intestine length (cm)	119.4	0.328	Karasov *et al.* (1986); ICPR (1992)
Total capilary surface area (cm^2)	26,810	10	Keele and Neil (1971)

TABLE 3
Physiological Data for Rabbits and Hamsters Used in the Model

Physiological parameter	Rabbit (body wt = 3.5 kg)	Hamster (body wt = 0.100 kg)
Blood volume (ml)	253	7.0
Organ weight (g)		
Liver	121	4.8
Kidneys	25	1.2
Lungs	31	1.0
Skin	420	17.1
Organ volume (ml)		
Others	2,386	62.0
Lumen volume (ml)		
Stomache	15	0.5
Small intestine	20	0.6
Blood flow (ml/min)		
Cardiac output	556	38.3
Liver, hepatic	25	1.2
Liver, splanchic	98	6.0
Kidneys	100	7.0
Lungs	13	0.7
Skin	38	2.6
Others	282	20.8
Clearance (ml/min)		
GFR	10	0.6
Small intestine		
length (cm)	180	56
Total capillary		
surface area (cm^2)	93,835	2681

Biliary excretion is simulated by a first-order rate mechanism from liver to GI tract. The first-order rate constants for biliary excretion are obtained by fitting and are kept constant for all animal species.

Physiological data and scaling. Many physiological parameters vary with body weight according to a power function.

$$Y = a \cdot \mathrm{BW}^b, \quad (1)$$

where the physiological variable Y equals a constant, a, times the body weight (BW) in kilograms raised to the power b, which is usually some fractional value smaller than 1.0. The scaling constants a and b are given in Table 2 and in the Appendix. The physiological values obtained by scaling according to the body weight for the hamsters and the rabbits are given in Table 3.

Parameters obtained by fitting. When no physiological parameters were available and calculation based on physicochemical properties was not feasible, the parameters required were obtained by fitting. Fitting of the model was done by simulation of experimental procedures, varying the unknown constants and visually comparing the graphs with experimental data. Table 4 gives a summary of the different studies used for fitting tissue affinity constants, absorption rate constants, and metabolic rate constants. These studies include data on rabbits and hamsters exposed to seven different arsenic compounds via three different routes of exposure (i.t., iv, and po) and at different doses. The experimental data available concern mainly the urinary and fecal excretion of the As metabolites 3 to 5 days after exposure. In some cases, limited results of tissue contents of the As metabolites are also reported.

The tissue affinity constants are fitted using data from studies on rabbits and hamsters, with the assumption that there are no species differences in the tissue affinities. They are kept constant for both species, as well as for humans (Mann *et al.*, 1996). On the other hand, the metabolic rate constants are fitted separately for each animal species because of the above-mentioned differences in the metabolic rates. The absorption rate constants from the GI tract to the liver for hamsters were fitted for each arsenic compound using four studies (studies 2, 3, 4, and 5 in Table 4). The absorption from the lungs to the plasma was obtained by comparing the model results with experimental data on hamsters exposed i.t. to arsenic. Absorption rates from the two lungs compartments (TB and P) are assumed to be the same.

RESULTS

Model Development

Fitting of the model. Several model parameters could not be obtained on a physiological or physicochemical basis and were therefore estimated by fitting the model results to experimental data. The tissue affinity constants, $K_{i,j}$ (tissue$_i$, metabolites $_j$), were estimated by fitting the tissue concentration of the As metabolites of the model to the experimental data for the rabbits and the hamsters. The $K_{i,j}$ obtained are given in Table 5. Metabolic rate constants were fitted for each animal species, using the excretion data of As metabolites in urine and feces. Only one study was used for rabbits

TABLE 4
Studies Used for Development of the Model

Study No.	Species	Intake route	As compound	Number of animals	Weight (kg)	Dose (μg As/kg body wt)	Reference
1	Rabbit	iv	$Na_3(AsO_4)$	13	3.5	400	Marafante *et al.* (1985)
2	Hamster	po, i.t.	$Na_3(AsO_4)$	4	0.115	2,000	Marafante *et al.* (1987)
			$NaAsO_2$	4	0.115	2,000	
			As_2S_3	4	0.115	2,000	
			$Pb_3(AsO_4)_2$	4	0.115	2,000	
3	Hamster	iv, po	As_2O_5	6	0.124	0.08	Charbonneau *et al.* (1980)
4	Hamster	po	As_2O_3	5	0.080	450	Yamauchi and Yamamura (1985)
5	Hamster	po	DMA	5	0.081	50,000	Yamauchi and Yamamura (1984)

TABLE 5
Tissue Affinity Constant Obtained by Fitting for Rabbits and Hamsters

	K_i			
Tissue (*i*)	As(V)	As(III)	MMA	DMA
Liver	1	200	10	1
Kidneys	40	20	100	5
Lungs	1	1	1	20
Skin	1	60	50	1
Others	10	40	1	1

(study 1, Table 4), while for hamsters the metabolic rate constants were fitted using all oral exposure experiments in study 2 (Table 4) in order to include exposure to As(III) and As(V). The estimates for the metabolic rate constants for both species are given in Table 6. The K_M estimated are much higher than the concentration reached in the liver for the highest exposure doses. Therefore methylation in the model is close to linearity and only the ratios V_M/K_M are relevant.

The last parameters that had to be estimated for the hamsters were the first-order absorption rate constants from the GI tract to the liver and from the lungs to the plasma. These were obtained by fitting using study 2 (Table 4), in which four different As compounds were given via two different exposure routes. For each As compound, an absorption rate was obtained. The corresponding predicted half-times for absorption from the GI tract to the liver and from the lungs to the plasma are summarized in Table 7.

Results for the rabbits. The experimental data used in the development of the model were taken from a study in which 13 rabbits were injected (iv) with [^{74}As]arsenate (Marafante *et al.*, 1985). The comparison of the model with the experimental data is shown in Fig. 2 and Table 8, the latter giving the amount of total As remaining in the tissues 6 and 24 hr after the injection.

TABLE 6
Metabolic Rate Constants Obtained by Separate Fitting for the Hamster and the Rabbit

Oxidation/reduction	First order	Rabbit	Hamster
Reduction	(1/hr)	3000	100
Oxidation	(1/hr)	6000	400
Kidney reduction	(1/hr)	30	1
Methylation	Michaelis–Menten		
1st step	$K_{M_{MMA}}$ (μmol/ml)	0.05	0.12
	$V_{M_{MMA}}$ (μmol/ml·hr)	4.0	0.12
2nd step	$K_{M_{DMA}}$ (μmol/ml)	0.9	0.08
	$V_{M_{DMA}}$ (μmol/ml·hr)	1.5	0.12

TABLE 7
Fitted GI Tract and Lung Absorption Half-Time (hr) for the Hamster

	Absorption half-time (hr)	
Exposure As compound	GI tract	Lung
As(V)		
$Na_3(AsO_4)$	0.08	12
$Pb_3(AsO_4)_2$	0.39	690
As_2O_5	0.28	—
As(III)		
$NaAsO_2$	0.08	12
As_2S_3	0.48	12
As_2O_3	0.02	—
DMA	0.09	—

Results for the hamsters. The first set of data used for the development of the model for the hamsters is found in the study by Yamauchi and Yamamura (1985), in which five hamsters were exposed to 450 μg As/kg body wt po of As_2O_3. The results of the simulation are shown in Fig. 3. Figures 3A and 3B represent the cumulative excretion of As metabolites, in urine and in feces, respectively. Figures 3C and 3D show the amount of As metabolites in the liver and kidneys 120 hr after the exposure.

Further comparisons of the model to experimental data were done using the study by Marafante and Vahter (1987). In this study, groups of four hamsters were exposed (po and i.t.) to 2000 μg As/kg body wt in the form of four different As compounds (Na_3AsO_4, $Pb_3(AsO_4)_2$, $NaAsO_2$, and As_2S_3). The simulation results presented in Fig. 4 represent the total body burden of As (BODY) and the cumulative excretion of total As in urine (UTOT) and feces (FTOT) after an oral administration. In Fig. 5, the same results are shown for the simulation of data for the i.t. instillation. The urinary metabolite distributions for both exposure routes are summarized in Table 9. Figure 6 shows simulation of a single oral dose.

Application

One of the interesting applications of PB-PK models is its use to study factors that may influence the kinetics, but are difficult to address experimentally. The model was applied to the description of several exposure scenarios in animals. The following features were studied as examples: chemical compounds (Na_3AsO_4, $Pb_3(AsO_4)_2$, and $NaAsO_2$) and animal species (rabbits and hamsters). Simulations were carried out for repeated exposure in order to demonstrate if accumulation of arsenic metabolites occurs. This is done with the

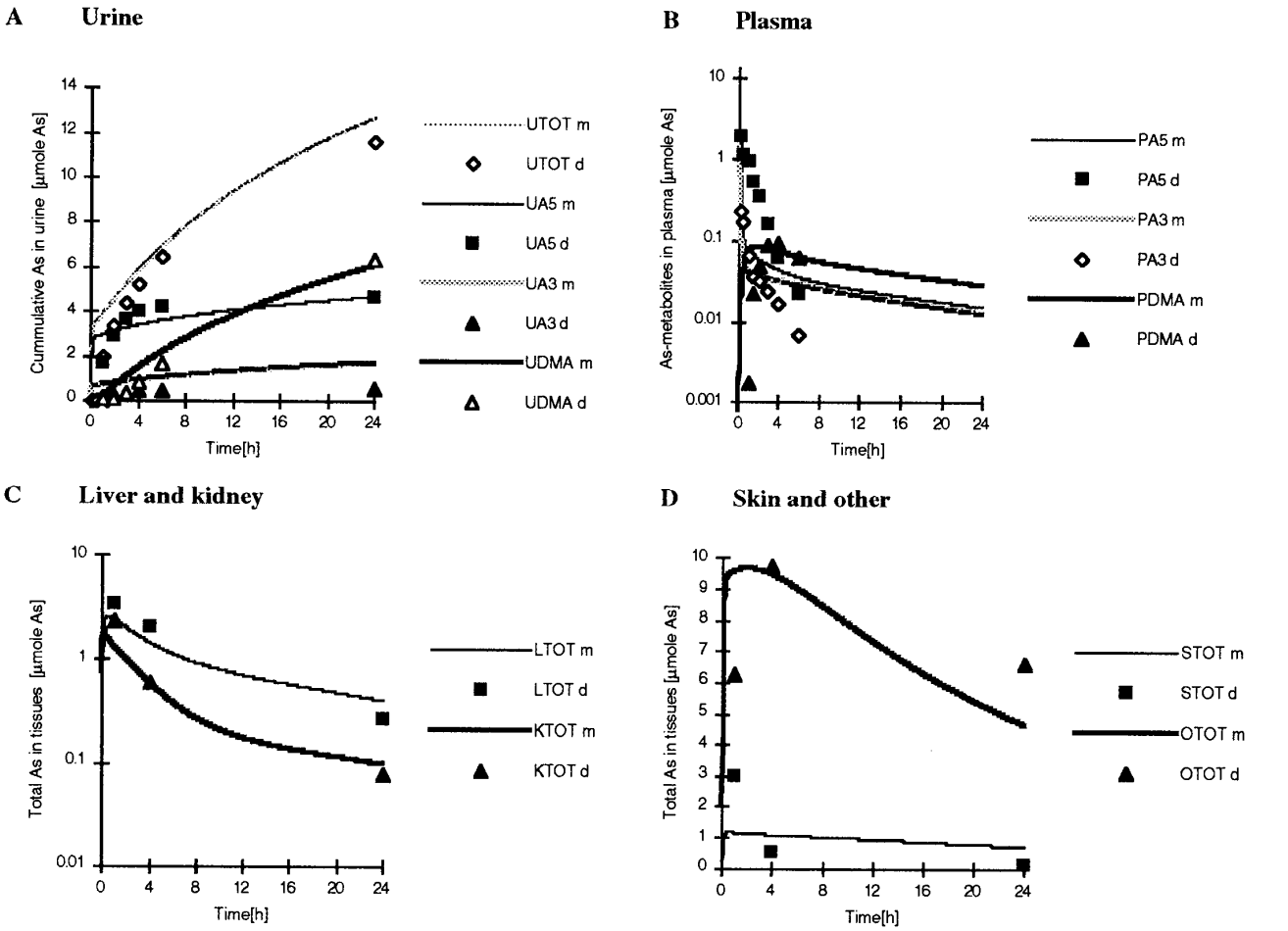

FIG. 2. Results of the simulation of an iv injection of 400 μg As/kg body wt of $Na_3(AsO_4)$ in rabbits. Experimental data are taken from Marafante *et al.* (1985). Graph A represents the cumulative urinary excretion of the As metabolites, graph B the amounts of the As metabolite quantities in the plasma, graph C the amounts of total As in the liver and kidney, and graph D the amounts of total As in the skin and other (m, model data; d, experimental data).

assumption that no changes in metabolism and distribution occur under repeated absorption.

Chemical compounds. The PB-PK model was used to simulate three repeated i.t. exposures (five doses: 1000 μg As/kg body wt) of Na_3AsO_4, $Pb_3(AsO_4)_2$, and $NaAsO_2$ in a hamster with a body weight of 0.1 kg. Figure 7 presents the results of the cumulative urinary excretion of the arsenic metabolites.

Animal species. The PB-PK model was used to compare simulation of a single oral exposure to Na_3AsO_4 (dose: 1000 μg As/kg BW) in a hamster (body weight = 0.1 kg) and in a rabbit (body weight = 3.5 kg). Figure 8 presents the simulation results of cumulative urinary excretion of the arsenic metabolites expressed as the percentage of the dose for both species.

TABLE 8

Comparison of Model Data with Experimental Data (Marafante *et al.*, 1985) for Total Amount of As in the Tissues (μmol As/Tissue) of Rabbits at Two Different Points in Time after an iv Injection of As(V) (dose: 400 μg As/kg body wt)

	Time (6 hr)		Time (24 hr)	
Tissue	Model	Experimental data	Model	Experimental data
Liver	1.13	2.03	0.41	0.27
Kidneys	0.38	0.59	0.10	0.08
Skin	1.06	0.50	0.72	0.11
Lungs	0.04	0.36	0.07	0.05
Other	9.00	9.75	4.63	6.59

DISCUSSION

Development of the Model

Rabbits. The experimental data used in the development of the model were taken from a study in which 13 rabbits were injected (iv) with [^{74}As]arsenate (Marafante

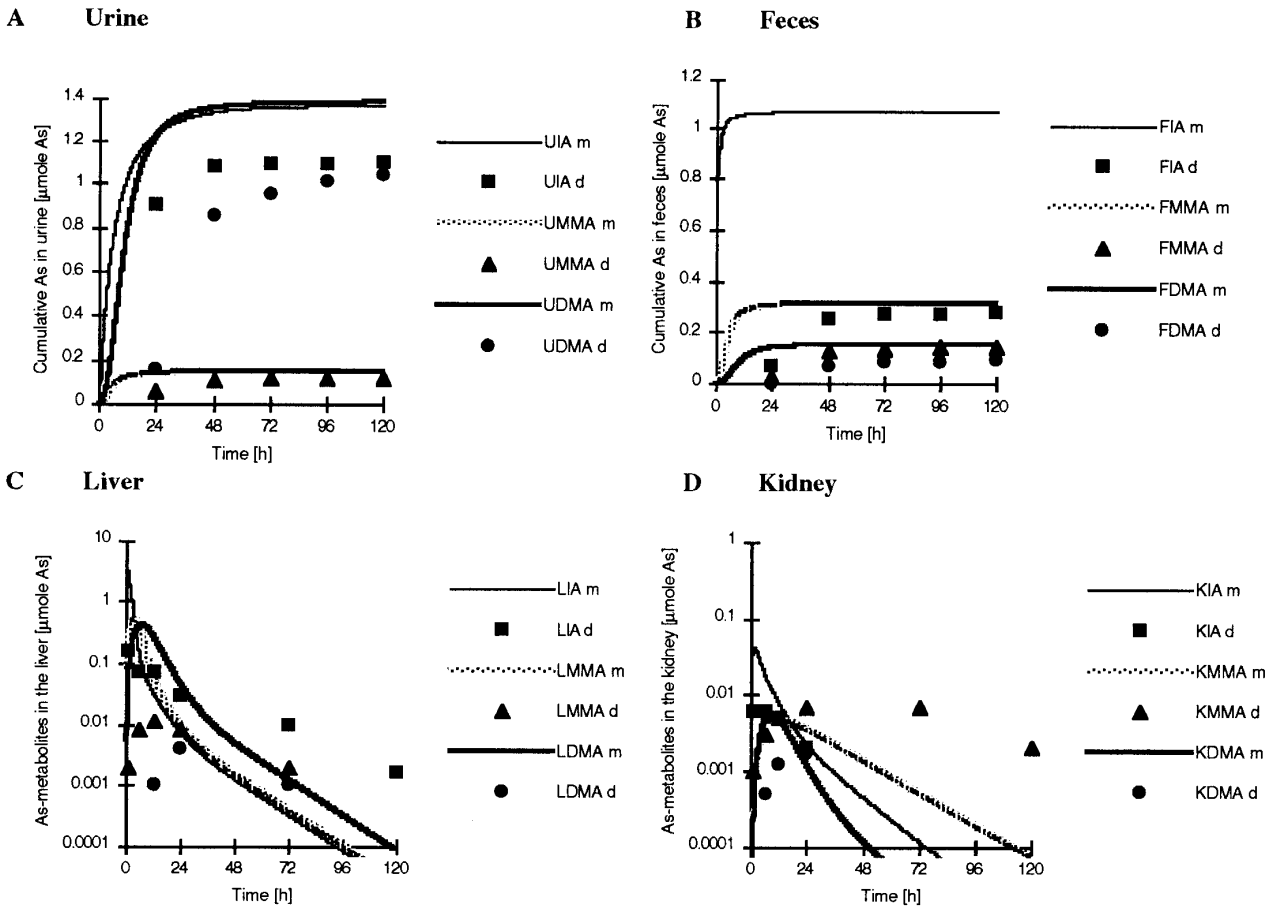

FIG. 3. Results of the simulation of a single oral administration of 450 μg As/kg body wet of As_2O_3 in hamsters. Experimental data are taken from Yamauchi and Yamamura (1985). Graphs A and B represent the cumulative urinary and fecal excretion of the As metabolites. Graphs C and D represent the amounts of the As metabolites in the liver and kidney (m, model data; d, experimental data).

et al., 1985). There is a good agreement between the model predictions and the experimental data (Table 8), except for the skin, for which the model data are too high. This is maybe due to a reduction of As(V) to As(III) lower than that assumed in the model, as As(III) is the form bound in the skin. Some discrepancy in the results appears in the period immediately following the injection (Fig. 2). Since renal clearance of As is proportional to the plasma concentration, urinary excretion of As(V) is very high during the first hour. This can be seen in Fig. 2A for the cumulative urinary excretion, where the As(V) amount in urine increases rapidly during the first hours after injection, and in the plasma (Fig. 2B), where the quantity of AsV decreases rapidly.

Hamsters. The first set of data used for the development of the model for the hamsters is found the study by Yamauchi and Yamamura (1985), in which five hamsters were exposed to 450 μg As/kg body wt po of As_2O_3. The model results for the urinary and fecal excretion (Fig. 3) are too high. This could be due to low experimental recoveries in feces and urine (estimated at 60%). On the other hand, the ratios for the metabolites in urine and feces obtained with the model are close to the experimental results. The simulation of inorganic As in the liver As metabolites is fairly good for the first 24 hr, but then the model decreases too fast. The model predicts too much of the two methylated metabolites during the first 24 hr after exposure. The quantity of As metabolites in the kidneys is well described by the model, with the exception of MMA, for which there is too fat a decrease after 24 hr.

Further comparison of the model to experimental data was done using the study by Marafante and Vahter (1987). In this study, groups of four hamsters were exposed (po and i.t.) to 2000 μg As/kg body wt to four different As compounds (Na_3AsO_4, $Pb_3(AsO_4)_2$, $NaAsO_2$, $NaAsO_2$, and As_2S_3). The situation following oral exposure (Fig. 4) is well stimulated for both As(V)

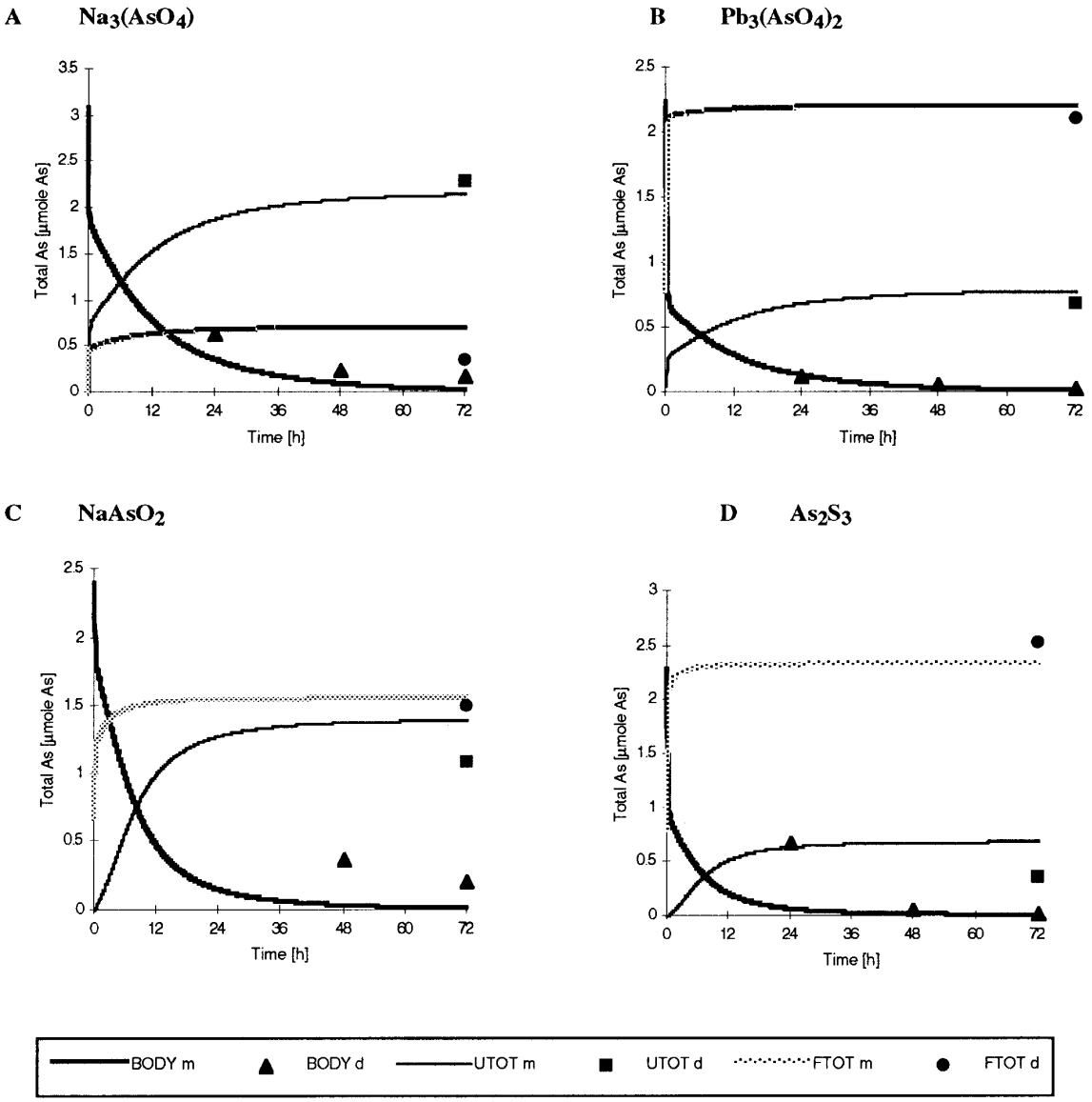

FIG. 4. Results of the simulation of a single oral administration of 2000 μg As/kg body wt of four As compounds in hamsters. Experimental data are taken from Marafante *et al.* (1987). The graphs represent the cumulative urinary and fecal excretion of total As and total amount of As in the body (m, model data; d, experimental data).

and As(III), although the body burden of total As decreases too fast after exposure to As(III) (Figs. 4C and 4D). The prediction of the distribution of the As metabolite in urine (Table 9) is very good for As(V) exposure and fairly good for As(III) exposure. The prediction of the response following i.t. exposure (Fig. 5) is again more satisfactory for exposure to As(V) than to As(III). The results of the As metabolite distribution in urine (Table 9) are good after i.t. instillation of both As(III) compounds and Na_3AsO_4, but the predicted amounts of DMA in urine after exposure to $Pb_3(AsO_4)_2$ are too low. There may be a reduction of As(V) already in the lungs due to the longer retention time.

Application

The PB-PK model was used to simulate three repeated i.t. exposure (five doses: 1000 μg As/kg body wt) of Na_3AsO_4, $Pb_3(AsO_4)_2$, and $NaAsO_2$ in a hamster with a body weight of 0.1 kg. The results of the simulation are given in Fig. 7.

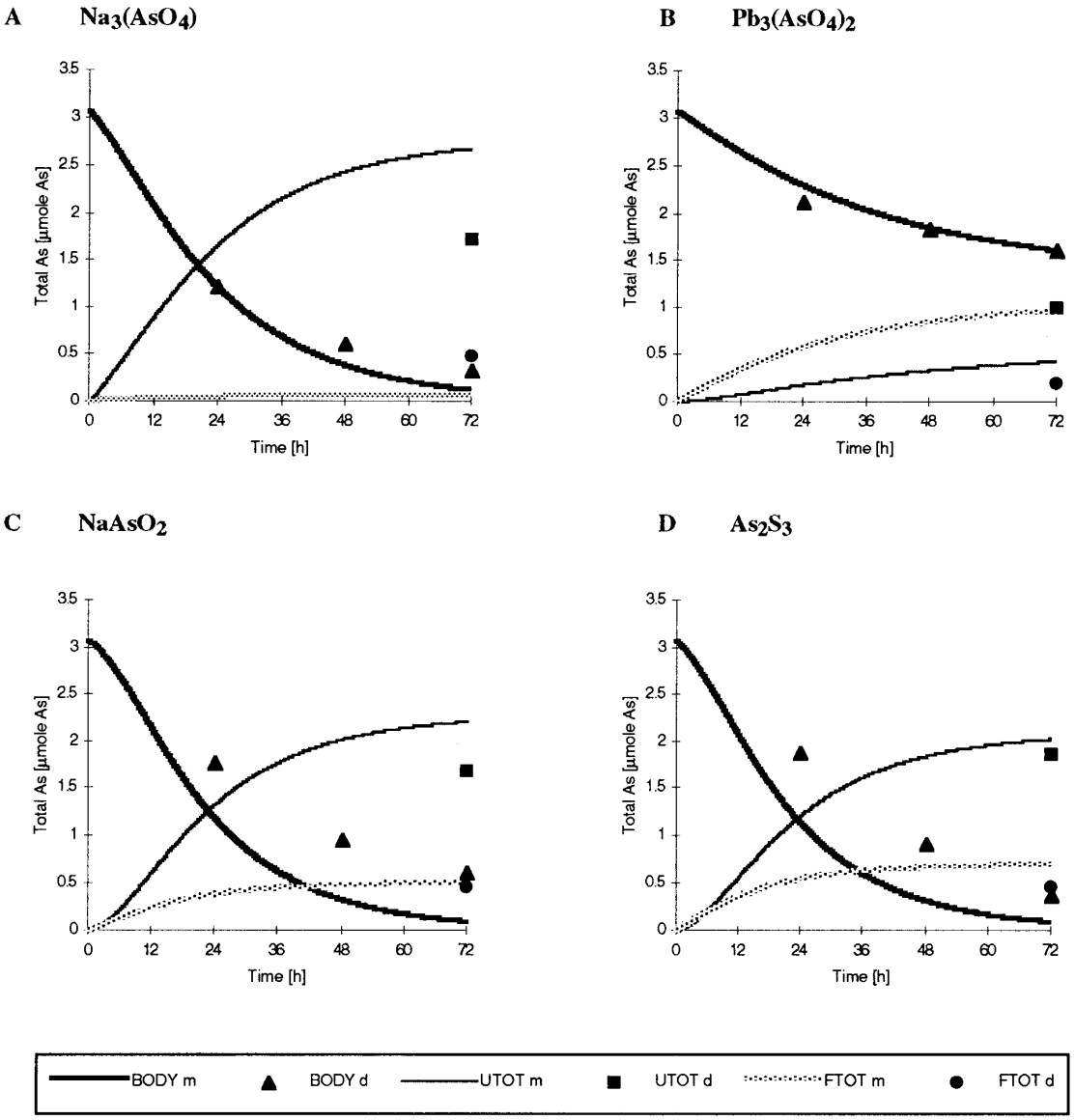

FIG. 5. Results of the simulation of a single i.t. instillation of 2000 μg As/kg body wt of four As compounds in hamsters. Experimental data are taken from Marafante *et al.* (1987). The graphs represent the cumulative urinary and fecal excretion of total As and the total amount of As in the body (m, model data; d, experimental data).

The predicted urinary excretions for $Pb_3(AsO_4)_2$ exposure, which is an arsenic compound with low solubility and low absorption rate in the lungs, show much lower arsenic content in urine for the four As metabolites. The predicted ratio of As(V) versus As(III) for $Pb_3(AsO_4)_2$ is the same as for Na_3AsO_4 (As(V)/As(III) = 3.5).This shows that the absorption rate in the lungs has a predictable influence on the total As urinary excretion for the same exposure dose, but has no influence on the As metabolite distribution in urine. For $NaAsO_2$ the As(V)/As(III) ratio is 1.6. The ratios between inorganic As and organic As are predicted to be 1.96 for As(V) exposure and 0.85 for As(III) exposure. The model predicts a higher methylation efficiency after exposure to As(III) than to As(V).

The PB-PK model was used to compare simulation of a single oral exposure to Na_3AsO_4 (dose: 1000 μg As/kg body wt) in a hamster (body weight = 0.1 kg) and in a rabbit (body weight = 3.5 kg). The results of the simulation are given in Fig. 8. The predicted total arsenic excreted in urine expressed as a percentage of the dose is

TABLE 9

Comparison of Model Data and Experimental Data on the Percentage of Urinary As Metabolites in Hamsters after Oral Exposure and i.t. Instillation (dose: 2000 μg As/kg body wt) of Different As Compounds (Marafante and Vahter, 1987)

Exposure route	Exposure compound	As(V)		As(III)		DMA	
		Experiment	Model	Experiment	Model	Experiment	Model
po	$Na_3(AsO_4)$	57	57	21	16	27	25
	$Pb_3(AsO_4)_2$	62	58	7	16	27	25
	$NaAsO_2$	46	30	30	19	25	47
	As_2S_3	10	32	21	20	64	45
i.t.	$Na_3(AsO_4)$	75	57	7	16	18	26
	$Pb_3(AsO_4)_2$	26	56	9	16	77	26
	$NaAsO_2$	20	19	33	12	49	64
	As_2S_3	9	16	9	10	77	69

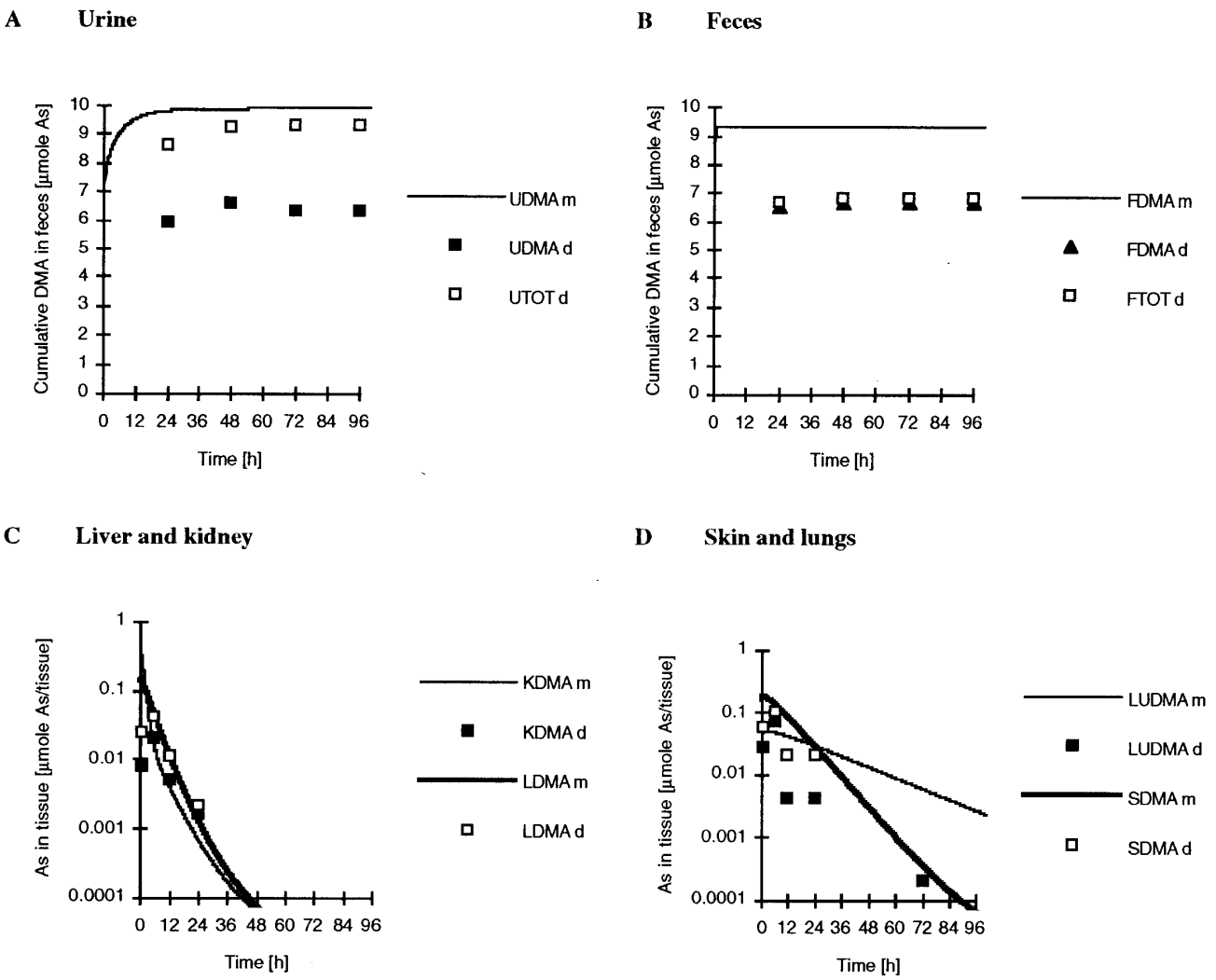

FIG. 6. Results of the simulation of a single oral administration of 17,778 μg As/kg body wt of DMA in hamsters. Experimental data are taken from Yamauchi and Yamamura (1984). The graphs represent the cumulative urinary and fecal excretion of DMA and the amount of DMA in the liver, kidney, skin, and lungs (m, model data; d, experimental data).

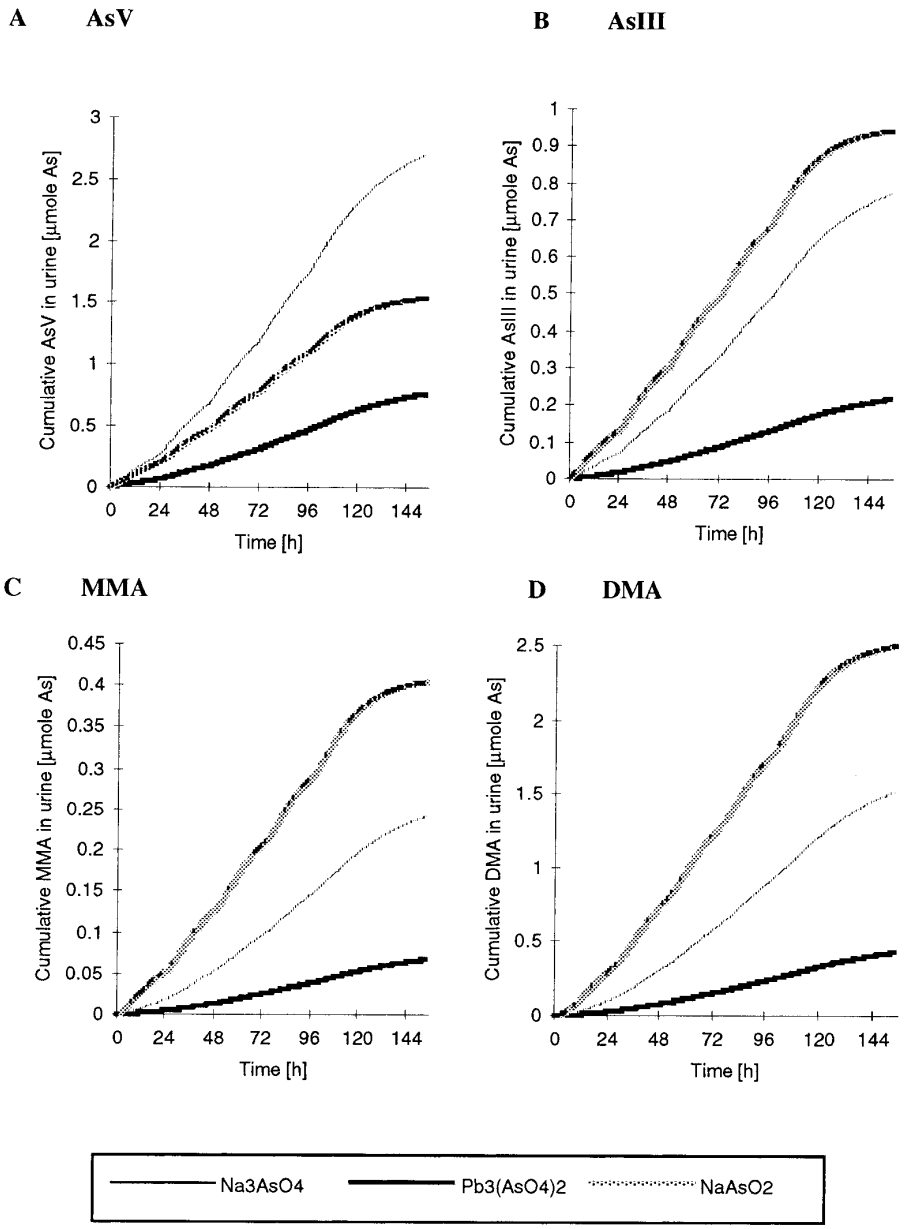

FIG. 7. Results of the simulation of repeated i.t. instillation of 1000 μg As/kg body wt of three As compounds, Na_3AsO_4, $Pb_3(AsO_4)_2$, and $NaAsO_2$, in hamsters (body wt = 0.1 kg). The graphs represent the cumulative urinary excretion of the As metabolites.

similar for both animal species during the first day. Within 4 days after the oral administration, the simulation for the rabbit predicts an excretion of 80% of the dose in urine, while for the hamster the predicted total As excretion in urine is only 60% of the dose. There is a distinct difference in the predicted As metabolite distribution in the urine between those two animal species. For rabbit, there is less than 1% excreted as As(V) and 50% as DMA, while for the hamster there is 35% excreted as As(V) and only 20% as DMA. The differences between the two species obtained by the model are related mainly to the methylation rates. This predicted variation in methylation efficiency should be considered in making interspecies comparison.

Limitations and Improvements

This PB-PK model is based on limited and sometimes uncertain experimental data. The results should therefore be interpreted with care. Also, caution should be exercised in

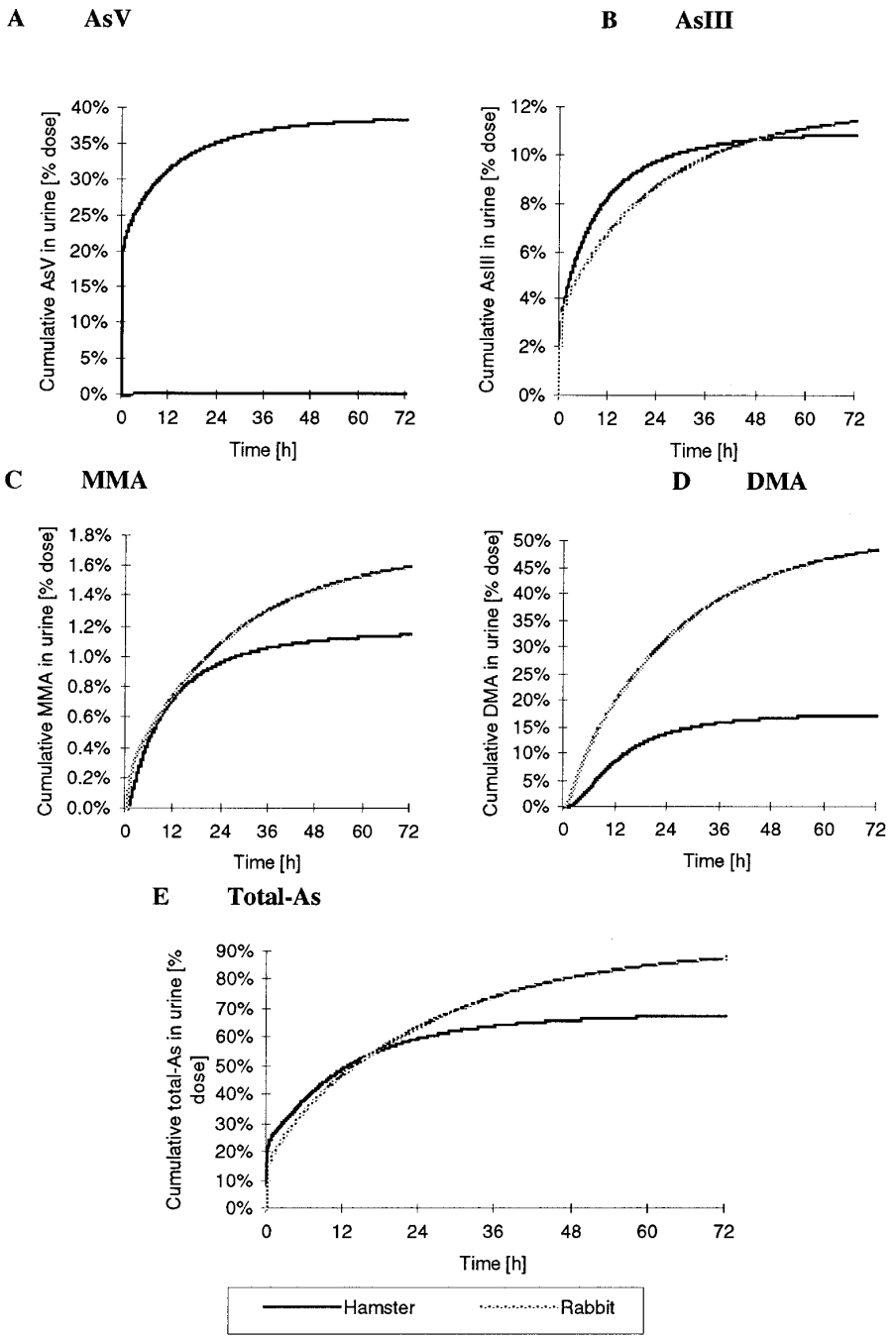

FIG. 8. Results of the simulation of a single oral administration of 100 μg As/kg body wt of Na_3AsO_4 in hamsters (body wt = 0.1 kg) and rabbits (body wt = 3.5 kg). The graphs represent the cumulative urinary excretion of the As metabolites expressed as the percentage of the dose for both species.

extrapolating to higher exposure levels because of uncertainties in the estimation of individual metabolic constants. In addition, experimental data for multiple dosing are needed to validate the predictions of the model for multiple dose exposure scenarios. The simulations are, however, promising and may be useful in their present state. Further improvements are planned to consolidate this model. In the present state, the model is useful in planning and interpretating animal studies.

Work is currently in progress to measure *in vitro* tissue affinity constants for the four As metabolites. This would reduce the number of fitted values, improve the physiolog-

ical sense of the model, and allow better estimation of the metabolic rates. Simulation of inhalation and oral exposure of humans will be presented in a subsequent paper (Mann *et al.*, 1996).

APPENDIX: LIST OF SYMBOLS

Symbol	Definition	Units
a	Scaling equation constant	—
b	Scaling equation power constant	—
BODY	Total As quantityin the body	μmol As
BW	Body weight	kg
D_p	Pore diffusion coefficient	cm^2/sec
$D_{p'}$	Restricted pore diffusion coefficient	cm^2/sec
FDMA	DMA quantity in feces	μmol As
FIA	As inorganic quantity in feces	μmol As
FMMA	MMA quantity in feces	μmol As
FTOT	Total As quantity in feces	μmol As
K	Tissue affinity constant	—
KDMA	DMA quantity in the kidney	μmol As
KIA	As inorganic quantity in the kidney	μmol As
$K_{M_{DMA}}$	Second methylation Michaelis–Menten constant	μmol As
KMMA	MMA quantity in the kidney	μmol As
$K_{M_{MMA}}$	First methylation Michaelis–Menten constant	μmol As
k_{ox}	Oxidation first-order rate constant	hr^{-1}
k_{red}	Reduction first-order rate constant	hr^{-1}
KTOT	Total As quantity in the kidney	μmol As
LDMA	DMA quantity in the liver	μmol As
LIA	As inorganic quantity in the liver	μmol As
LMMA	MMA quantity in the liver	μmol As
LTOT	Total As quantity in the liver	μmol As
LUDMA	DMA quantity in the lung	μmol As
M_r	Molecular radius	Å
OTOT	Total As quantity in the other tissue	μmol As
P	Permeability	cm/hr
PA3	As(III) quantity in the plasma	μmol As
PA5	As(V) quantity in the plasma	μmol As
PDMA	DMA quantity in the plasma	μmol As
P_r	Pore radius	Å
Q	Blood flow	ml/hr
q	Quantity of As	μmol As
SC	Capillary surface	cm^2
SDMA	DMA quantity in the skin	μmol As
SP	Pore surface	cm^2
STOT	Total As quantity in skin	μmol As
TCS	Total capillary surface	cm^2
UA3	Cumulative As(III) quantity in urine	μmol As
UA5	Cumulative As(V) quantity in urine	μmol As
UDMA	Cumulative MMA quantity in urine	μmol As
UIA	Cumlative As inorganic quantity in urine	μmol As
UMMA	Cumulative DMA quantity in urine	μmol As
UTOT	Cumulative total As quantity in urine	μmol As
V	Volume	ml
$V_{M_{DMA}}$	Second methylation Michaelis–Menten maximum rate constant	μmol/hr
$V_{M_{MMA}}$	First methylation Michaelis–Menten maximum rate constant	μmol/hr
Y	Physiological variable	g

Subscripts

art	Arterial
i	Tissue
int	Interstital
j	As compound
pl	Plasma
t	Tissue
ven	Venous
′	Apparent distribution

Mathematical Equations

This section describes the mathematical equations used in the model for the distribution of the As metabolites in tissues [Eq. (A4) and (A8)] and plasma [Eq. (A11)]. For each As metabolite and each distribution compartment, one equation must be writen. Then each distribution equation must be completed with the absorption equations, metabolic equations, and excretion equations.

Transfer across the capillary membranes is considered to be due to diffusion through the pores for As(V), MMA, and DMA, which are ionized mostly at physiological pH, with pK_{a1} of 1.2, 3.6, and 6.2, respectively (Vahter and Marafante, 1993; HCP, 1978–79). As(III), with pK_{a1} of 9.2 (HCP, 1978–79), is nonionic at physiological pH and, therefore, diffusion takes place across the entire capillary area.

Equation for the transfert surface area. The total capillary surface area (TCS) is calculated using a scaling equation based on the muscle weight expressed in kg (Keele and Neil, 1971):

$$\text{TCS (cm}^2) = \text{muscle weight} \cdot 70{,}000. \qquad \text{(A1)}$$

The tissue capillary surface area (SC_i) is based on the ratio of the blood flow (Q_i) and the cardiac output:

$$SC_i\ (\text{cm}^2) = \text{TCS} \cdot \frac{Q_i}{\text{cardiac output}}. \qquad \text{(A2)}$$

Total pore area (SP_i) is calculated as the fraction of the capillary occupied by the pores times the tissue capillary surface area of the tissue:

$$SP_i\ (\text{cm}^2) = SC_i \cdot \frac{\%\ \text{of pore area}_i}{100}. \qquad \text{(A3)}$$

The thickness of the capillaries was set to 1 μm for discontinuous and fenestrated capillaries (Snyder *et al.*, 1992). Taking into account that the continuous capillaries consist of a single layer of endothelial cells and that the other capillaries consist of a thin endothelial layer (Keel and Neil, 1971), the thickness of the continuous capillaries was set to 0.5 μm.

Distribution equation for ionic compounds (As(V), MMA, and DMA).

$$\frac{dq_{i,j}}{dt}\ (\mu\text{mol/hr}) = \frac{P_{i,j} \cdot SP_i \cdot \left(\frac{q_{\text{pl},j}}{V_{\text{pl}}} - \frac{q_{i,j}}{V'_i}\right)}{1 + \frac{P_{i,j} \cdot \text{SP}_i}{Q_{\text{pl},i}}}, \quad \text{(A4)}$$

where the permeability constant is calculated as

$$P_{i,j}\ (\text{cm/hr}) = \frac{D'_{\text{p}} \cdot 360{,}000}{\text{capillary thickness,}} \quad \text{(A5)}$$

with the pore diffusion coefficient (D_{p}) (Berner and Cooper, 1985) as

$$D_{\text{p}}\ (\text{cm}^2/\text{sec}) = 3.8 \times 10^{-5} \exp(-0.016 \cdot M_{\text{W}}) \quad \text{(A6)}$$

and the restricted diffusion coefficient (D'_{p}) (Keele and Neil, 1971) as

$$D'_{\text{p}}\ (\text{cm}^2/\text{sec}) = D_{\text{p}} \cdot \left(1 - \frac{M_{\text{r}}}{P_{\text{r}}}\right)^2 \cdot [1 - 2.10 \cdot (M_{\text{r}}/P_{\text{r}}) + 2.09 \cdot (M_{\text{r}}/P_{\text{r}})^3 - 0.95 \cdot (M_{\text{r}}/P_{\text{r}})^5]. \quad \text{(A7)}$$

Distribution equation for As(III) (nonionic compound).

$$\frac{dq_{i,j}}{dt}\ (\mu\text{mol/hr}) = \frac{P_{i,j} \cdot \text{SC}_i \cdot \left(\frac{q_{\text{pl},j}}{V_{\text{pl}}} - \frac{q_{i,j}}{V'_i}\right)}{1 + \frac{P_{i,j} \cdot \text{SC}_i}{Q_{\text{pl},i}}}, \quad \text{(A8)}$$

where the permeability constant is calculated as

$$P_{i,j}\ (\text{cm/hr}) = \frac{D_{\text{c}} \cdot 360{,}000}{\text{capillary thickness}}, \quad \text{(A9)}$$

with the capillary diffusion coefficient (D_{c}) (Berner and Cooper, 1985) as

$$D_{\text{c}}\ (\text{cm}^2/\text{sec}) = 1.7 \times 10^{-5} \exp(-0.016 \cdot M_{\text{W}}). \quad \text{(A10)}$$

Distribution equation for the plasma. Equation (A11) represents only the distribution for the plasma without the metabolic, absorption, and excretion equations:

$$\frac{dq_{\text{pl},j}}{dt}\ (\mu\text{mol/hr}) = \sum_{i=\text{liver}}^{\text{tissue}} - \frac{dq_{i,j}}{dt}. \quad \text{(A11)}$$

Software Considerations

The mathematical model of the PB-PK model was performed with Simusolv version 2.1, Dow Chemical Co. The Simusolv computer program is an integrated, multifunctional software package designed to help scientists and engineers to develop and use mathematical models of dynamic physical systems.

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