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Pharmacokinetics and Expert Systems as Aids for Risk Assessment in Reproductive Toxicology

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A minimal approach to risk assessment in reproductive toxicology involves four components: hazard identification, hazard characterization, exposure characterization, and risk characterization. In practice, risk assessment in reproductive toxicology has been reduced to arbitrary safety factors or mathematical models of the dose-response relationship. These approaches obscure biological differences across species rather than using this important and frequently accessible information. Two approaches that are formally capable of using biologically relevant information (pharmacokinetics and expert system shells) are explored as aids to risk assessment in reproductive toxicology.

Introduction

In spite of gaps in our knowledge of reproductive vulnerability to xenobiotics across species (*1*), data and theoretical approaches are available that would allow more rational and consistent prediction of human reproductive risk. In addition, *in vivo* and *in vitro* experimental models are available that allow refinement of those predictions. This discussion will explore the utility of pharmacokinetics and expert systems as scientific and regulatory tools in reproductive risk assessment.

Reproductive Risk Assessment

At the present time, risk assessment in reproductive toxicology is conducted by most regulatory agencies using safety factors, multiples of 10^{-1} used to adjust a defined dose or no-observed-effect-level in an experimental animal model to the permissible level for human exposure (*2*) (Fig. 1). One example of recommended safety factors for teratogenicity that uses this factor of 10 approach has been proposed by Wilson and modified by Schardein (Table 1). This approach suggests that for drugs with a high benefit-to-risk ratio, doses similar to those producing teratogenicity or embryotoxicity may be used if clinically indicated. For food additives or pesticides, a safety factor of 100, and for environmental pollutants, a safety factor of 1000, is suggested.

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Although the safe level in this factor of 10 approach is defined by exposure or use, it is independent of species differences in anatomy, physiology, pharmacology, and toxicology. Even worse, a factor of 10 approach to permissible human exposure is doomed to overregulate xenobiotics that are false positives and allows excessive human exposure to false negatives, adding to social costs and impairing human reproductive health.

Available Data on Reproductive Hazards

In their review, Barlow and Sullivan (*1*) collected animal and human data on 48 chemicals of industrial interest (Table 2). All 48 chemicals had relevant pharmacology and toxicology data, 38 were positive, producing adverse effects in experimental animals, and 10 were negative. In no case were the data from experimental animal studies insufficient for assigning the chemical to a group, either positive or negative. In humans, there were sufficient pharmacologic and toxicologic data on only 19 of the 48 chemicals, all positive. The remaining 29 studies had insufficient data concerning human pharmacology and toxicity.

The authors then evaluated the available data from animals and humans addressing reproductive toxicity; endocrine or gonadal effects, alteration in fertility, and effects on pregnancy.

Among the 48 chemicals, 11 had data suggesting adverse gonadal effects in male or female experimental animals, 4 were negative in males, and 2 were negative in females. Most of the chemicals, however, had insufficient data to define gonadal toxicity in experimental animals or humans. Xenobiotic effects on fertility were also poorly characterized, with no animal data on 33 or 34 compounds, and insufficient human data on 45 or 46

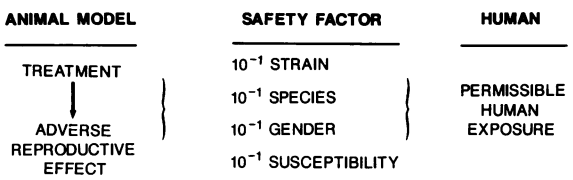

FIGURE 1. Current approach to risk assessment in reproductive toxicology.

chemicals. Although there is a bit more data on the effects of these 48 chemicals on pregnancy in experimental animals, human data are clearly lacking.

This suggests two factors: For many chemicals, animal data necessary to define reproductive hazard are not available, and where animal data are available, even minimal human data are lacking. Therefore, once a reproductive hazard has been identified, extrapolation across species will be necessary to define human risk.

Extrapolation Across Species

The necessity to develop better methods of predicting human reproductive risk from animal data is illustrated for teratogenicity (Table 3) (*3*). This table summarizes a review conducted by Frankos of xenobiotics classified as human teratogens (38 drugs and chemicals) and 165 xenobiotics not hazardous during pregnancy (*3*). Note that not all reproductive toxicologists would agree that there are 38 known human teratogens; however, it is instructive to examine the table for its meaning (see Table 4 for a list of animal and human teratogens).

Table 1. Suggested safe levels of different chemicals with teratogenic potential.[a]

Chemical class	Embryotoxicity/usage ratio
Drugs with high benefit/risk ratio	1:>1
Drugs (general)	1:1 to 1:0.1
Food additives, pesticides	1:0.01
Air, water, and food pollutants	1:0.001

[a] From Schardein (*2*).

Table 2. Reproductive hazards of industrial chemicals.[a]

	Pharmacology and toxicology	Endocrine and gonadal		Fertility		Pregnancy
		M	F	M	F	
Animal						
Positive[b]	38	11	11	9	9	17
Negative[c]	10	4	2	6	5	9
No data[d]	0	33	35	33	34	22
Human						
Positive	19	8	6	2	1	6
Negative	0	0	3	1	1	1
No data	29	40	39	45	46	41

[a] From Barlow and Sullivan (*1*).
[b] Positive, studies performed suggest toxicity.
[c] Negative, studies performed suggest no toxicity.
[d] No data, no studies or inconclusive.

Table 3. Animal models as predictors of human teratogens.[a]

	Mouse	Rat	Rabbit	Hamster	Monkey
Positive (38)[b]	85%+	80%+	60%+	45%+	30%+
Negative (165)[c]	35%−	50%−	70%−	35%−	80%−

[a] Positive and negative compounds not specified. From Frankos (*3*).
[b] Positive, reports of birth defects in humans.
[c] Negative, known not to be human teratogens.

Of those xenobiotics positive for reproductive toxicity in humans, 85% were positive in the mouse, and 80% were positive in the rat. In the rabbit, hamster, and monkey, 60, 45, and 30% were positive, respectively. The species with the best record in identifying human teratogens, the mouse, was positive for 85% of xenobiotics thought to be human teratogens. Among those xenobiotics that were not human teratogens, only 35% were correspondingly negative in the mouse and the hamster. In the rat, rabbit, and monkey, 50, 70, and 80% were negative, respectively. This disparity indicates that it is essential to develop better predictors. Mechanism-based physiological-pharmacokinetic models and expert systems for defining human reproductive risk appear appropriate.

Approach to Risk Assessment in Reproductive Toxicology

Conducting risk analysis using safety factors does provide a level for permissible human exposure; unfortunately, this number cannot be adjusted by advances in reproductive physiology, pharmacology, or toxicology. In addition, using safety factors to set permissible human exposures is unacceptable because the uncertainty is completely undefined. A more rational approach for risk assessment would be to determine the physiological, pharmacological, toxicological, cellular, and molecular characteristics that control reproductive toxicity in experimental models and translate that information into predicted human risk based on similar human characteristics (Fig. 2).

Three specific areas need to be addressed to develop risk assessment in reproductive toxicology. The first area is gathering data on reproductive physiology and the reproductive effects of a broad range of chemicals across species. One component of this is development of data bases that encourage efficient use of previously collected information on reproductive physiology, pharmacology, and toxicology.

The second area of need is methods for translating information on reproductive hazards in experimental animals into risk assessments that protect human populations.

Finally, the third area of need is experimental and theoretical models that can define the site and mechanism of action of reproductive toxins (*4*).

Teratogenicity Across Species

One of the major limitations in conducting risk assessments in reproductive toxicology is the diversity of

Table 4. *In vivo* teratology data: Comparison across species.[a]

Compound	Mouse	Rat	Rabbit	Monkey	Human	Rule-based estimate of risk		
						S	SS	P
Chloroambucil	+	+			+	H	H	H
Coumarin	−		+		+	M	L	M
Cyclophosphamide	+	+	+	+	+	H	H	H
Diazepam	+	−			+	M	M	M
Diethylstilbestrol	+	+		+	+	H	H	H
Diphenylhydantoin	+	+	+		+	H	H	H
Ethanol	+	+			+	H	H	H
5-Fluorouracil	+	+		+	+	H	H	H
Meprobamate	+	+	−		+	H	H	H
Methotrexate	+	+	+	+	+	H	H	H
Methylmercury Cl	+	+			+	H	H	H
L-Phenylalanine				+	+	M	M	H
Procarbazine HCl	+	+	+		+	H	H	H
13-*cis*-Retinoic acid	+	+			+	H	H	H
Testosterone proprionate		+		+	+	H	M	H
Thalidomide	−	−	+	+	+	M	M	H
Acetylsalicylic acid	+	+		+	−	H	H	H
Caffeine	+	+	+		−	H	H	H
Diphenhydramine HCl	−	−	−		−	L	L	L
Doxylamine succinate	−	−		−	−	M	L	L
Isoniazide	−	−	−		−	L	L	L
Penicillin G	−		−		−	M	L	M
Saccharin	−	−	−		−	L	L	L
2,4,5-T	+	−		−	−	M	M	M

Abbreviations: S, sum rules; SS, sum of square rules; P, primate rules; H, high risk for human teratogenicity; M, moderate risk for human teratogenicity; L, low risk for human teratogenicity.

[a] Data from Schardein (*2*), Frankos (*3*), Smith et al. (*5*), and Shepard (*6*).

reproductive physiology across species. This diversity suggests that for extrapolation across species, risk assessments require access to data detailing species differences in reproductive physiology, pharmacology, and toxicology (Table 4). Note that Table 4 lists only 16 compounds as positive human teratogens, fewer than the 38 compounds indicated in Table 3. Among those 16 compounds thought to be human teratogens, 12 were also teratogenic in all other species tested. Among the 8 that are thought not to be teratogenic, 5 were negative in all species tested. Discordance in the outcome across species for a given reproductive end point suggests that hazard identification, the first step in risk analysis, is not a trivial process. Clearly, a more detailed analysis of animal responses to reproductive toxins will be necessary before quantitative risk assessment can be performed. Our discussion of expert systems is designed to explore one approach to this problem.

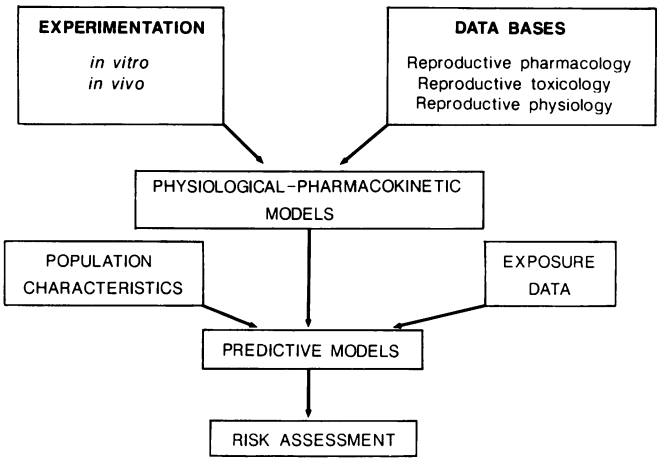

FIGURE 2. Suggested approach to reproductive risk assessment.

Because of past research interests in reproductive medicine, a considerable amount of data are available on maternal, placental, and fetal physiological parameters for humans, nonhuman primates, and sheep. In addition, a large amount of toxicological data exploring embryonic and fetal effects of xenobiotics are available for rodents. This physiological, pharmacological, and toxicological data should be collected into a computerized data base and made available to interested investigators. To demonstrate the utility of such a data base we will also review the impact of physiological changes during human pregnancy on xenobiotic uptake, distribution, metabolism, and excretion.

Expert Systems

Physicians are frequently confronted with questions concerning the effects of a drug or chemical on human reproduction. As previously indicated (Table 2) there are generally insufficient data to identify the drug or chemical as a reproductive toxin for humans. For this reason it is almost always necessary to extrapolate reproductive toxicity data across species to arrive at a prediction of the likelihood of human hazard and estimate the degree of human risk.

Table 5. Structure of ESIE rule-based expert system shell.

Process	Number allowed
Goal: Human teratogenicity	1
Legal answers: Yes, no, ?	50
Rules (See Table 7)	400
Questions	100
Answer: The risk for human teratogenicity is	1

Extrapolation across species to define human risk is a difficult process that requires knowledge of species differences in reproductive biology, development, pharmacology, and toxicology, all highly specialized disciplines. Because of the complex nature of knowledge required to make a rational judgment of human risk, the utility of an expert system shell has been explored for risk assessment in reproductive and developmental toxicology.

Expert System Shell

The expert system shell used was ESIE (Expert System Inference Engine, Lightway Consultants, Tampa, FL). ESIE is a rule-based expert system shell written in PASCAL for MS-DOS computers. The expert system shell is first given a goal; in this case the goal is to determine the relative risk for human teratogenicity (Table 5). The software then is given the allowable answers ($\leq$ 50) to the questions. In this example the allowable answers to questions concerning teratogenicity were: yes, the compound is teratogenic; no, the compound does not produce teratogenicity; or ?, the teratogenicity of the compound is underfined either because of the lack of data or the presence of conflicting data. The rules that determine how responses to questions are treated are then defined. This expert system shell is capable of using up to 400 rules.

The expert system shell can then be programmed to request information by asking up to 100 questions. The response to these questions must be in the format of a legal answer (e.g., yes, no, ?). This information is then interpreted with the rules.

Animal Data

Because of the limitation on the number of rules ($\leq$ 400) that can be used in ESIE, it was decided to limit the number of species of animals for which teratogenicity information was requested. In order to determine which experimental animals would be useful to include in the rules written for ESIE, a survey of animals used in teratogenicity testing was conducted (Table 6) using a recently published reference (*2*). According to Schardein, approximately 1528 drugs and 1252 chemicals have been evaluated for teratogenicity in experimental animals.

Among the 1528 drugs that have been tested for teratogenicity, 1124 (74%) were tested in rats, 686 (45%) were tested in mice, and 566 (37%) were tested in rabbits. Among the 1252 chemicals tested for teratogenicity, 862 (69%) were tested in rats, 435 (35%) were tested in mice, and 196 (16%) were tested in rabbits. These data suggest that including rules concerning the teratogenicity of a compound in rats, mice, and rabbits will include the experimental data available for many compounds. In addition, based on cost and regulatory considerations, there is little reason to think that the use of these species for teratogenicity testing will change in the near future.

Table 6. Teratogenicity testing in experimental animals.[a]

	Drugs		Chemicals	
Species	Number	%	Number	%
Rat	1124	74	862	69
Mouse	686	45	435	35
Rabbit	566	37	196	16
Hamster	110	7	111	9
Dog	52	3	17	1
Pig	35	2	31	2
Sheep	33	2	39	3
Guinea pig	39	3	23	2
Cat	10	<1	5	<1
Ferret	8	<1	2	<1
Cow	13	<1	33	3
Horse	2	<1	4	<1
Primate	82	5	24	2
Total	1528		1252	

[a] This table lists drugs and chemicals that have been studied for teratogenicity. Note that some drugs or chemicals were tested in more than one experimental animal model.

Expert System Rules

The rules used in this expert system are shown in Table 7. The rules and their corresponding questions are closely linked. The three questions asked are: Is the compound teratogenic in rats? Is the compound teratogenic in mice? and Is the compound teratogenic in rabbits?

The difficult part of this expert system, as indeed the most complex part of any expert system, is actually defining the rules. That is, how should a given set of animal outcomes be evaluated with respect to human hazard? In this implementation of the expert system, information was requested by questions on the teratogenicity in three species: rat, mouse, and rabbit. If the compound had been tested and was known to be positive, that is, if it produces malformations or fetal death at doses below those associated with maternal toxicity, the answer is "yes," if negative, the answer is "no." If, however, the compound had not been tested, or if the data were difficult to interpret or contradictory, the answer is "?".

There are 27 different combinations of these allowable answers among the three species (Table 7). Two ways were initially used to define the human teratogenicity of a given combination. In both cases the allowable answers were given a numerical score: no = 1, ? = 2, yes = 3. In the first method (sum rule) the answers were summed and the human risk determined as defined on Table 8. A second rule was also explored—the numerical

Table 7. Rules used in expert systems for teratogenicity.

Mouse	Rat	Rabbit	Sum	Sum of squares
No(1)	No(1)	No(1)	3	3
No(1)	No(1)	?(2)	4	6
No(1)	No(1)	Yes(3)	5	11
No(1)	?(2)	No(1)	4	6
No(1)	?(2)	?(2)	5	9
No(1)	?(2)	Yes(3)	6	14
No(1)	Yes(3)	No(1)	5	11
No(1)	Yes(3)	?(2)	6	14
No(1)	Yes(3)	Yes(3)	7	19
?(2)	No(1)	No(1)	4	6
?(2)	No(1)	?(2)	5	9
?(2)	No(1)	Yes(3)	6	14
?(2)	?(2)	No(1)	5	9
?(2)	?(2)	?(2)	6	12
?(2)	?(2)	Yes(3)	7	17
?(2)	Yes(3)	No(1)	6	14
?(2)	Yes(3)	?(2)	7	17
?(2)	Yes(3)	Yes(3)	8	22
Yes(3)	No(1)	No(1)	5	11
Yes(3)	No(1)	?(2)	6	14
Yes(3)	No(1)	Yes(3)	7	19
Yes(3)	?(2)	No(1)	6	14
Yes(3)	?(2)	?(2)	7	17
Yes(3)	?(2)	Yes(3)	8	22
Yes(3)	Yes(3)	No(1)	7	19
Yes(3)	Yes(3)	?(2)	8	22
Yes(3)	Yes(3)	Yes(3)	9	27

Table 8. Sum rules for predicting human teratogenicity using mouse, rat, and rabbit data.

Sum	Human teratogenic risk
3	Low
4 to 6	Moderate
7 to 9	High

Table 9. Sum of squares rule for predicting human teratogenicity using mouse, rat, and rabbit data.

Sum of squares	Human teratogenic risk
≤ 6	Low
>6 to <19	Moderate
≥ 19	High

scores were squared before summing. This second approach has the advantage of weighing positive and uncertain data more than negative data. Using the sum of squares method, human risk for teratogenicity was determined as defined on Table 9.

In addition, a third set of rules was derived by including data on teratogenicity testing in nonhuman primates. The structure of this third set of rules was similar to the sum of squares rules with the exception that teratogenicity in a nonhuman primate was considered to represent high teratogenic risk for humans.

Testing the Rules with Human and Animal Data

Using the three sets of rules, it is possible to explore predictability using compounds defined as teratogenic or nonteratogenic in humans (Table 4). The results of using these three sets of rules to evaluate human teratogenic risk with these data are illustrated in Table 10.

Using the sum rule, 12 of the 16 compounds that are teratogenic in humans were identified as having high teratogenic risk. The remaining 4 of the 16 were classified as moderate human teratogenic risks. The sum of squares rule classified 11 of 16 as high human teratogenic risk among those identified as human teratogens. Four were classified as posing moderate risk and one as low human teratogenic risk. The third rule set using primate data classified 14 of 16 as representing high human teratogenic risk and 2 as moderate teratogenic risk.

Finally, among the 8 compounds identified as nonteratogenic for humans, the sum, sum of squares, and primate rules classified 2 as high risk. Three were classified as moderate risk using the sum, 2 using the primate, and 1 using the sum of squares rule. The classification of low risk was applied to 3 compounds using the sum, 5 using the sum of squares, and 4 using the primate rules.

More complex rules containing information on testing in other species, dose-response relationships, and types of malformations can be developed and evaluated in this and other expert system shells. The utility of this approach is the consistent definition of reproductive hazard. Where human experts disagree on the classification of a compound, the disagreement can be used to design experiments that enhance the rules used in the decision-making process. Note that these rule-based expert systems will initially have greatest utility in hazard identification and qualitative risk assessment. Quantitative risk assessment will need to be based on physiological and pharmacokinetic models.

Maternal Physiological Alterations During Pregnancy

Complex alterations in maternal pulmonary, cardiovascular, renal, gastrointestinal, and hepatic function occur during pregnancy (*7,8*). These physiological changes during pregnancy may alter the uptake, distribution, metabolism, or clearance of xenobiotics by the pregnant woman, placenta, and fetus (*9–12*). Physiological alterations during pregnancy may also alter maternal response to environmental toxins. Any method for quantitative risk assessment that includes extrapolation across treatment, route, or species for maternal, placental, or fetal toxicity must consider these physiological adaptations.

Absorption

During pregnancy there are physiological changes in several systems that can alter the rate and amount of a xenobiotic absorbed. Intestinal motility is decreased and gastric emptying time is increased during pregnancy (*13*). This means that xenobiotics will spend a

Table 10. Prediction of human teratogenic risk using an expert system shell.

	Expert system rule								
	Sum			Sum of squares			Primate teratogenicity		
Human teratogenicity	High	Moderate	Low	High	Moderate	Low	High	Moderate	Low
Teratogenic	12	4	0	11	4	1	14	2	0
Nonteratogenic	2	3	3	2	1	5	2	2	4

longer time in both the stomach and the small intestine. If the xenobiotic is absorbed through the small intestine, increased residence time in the stomach may delay the time to peak concentration in maternal and fetal compartments. In addition, the xenobiotic may be metabolized in the stomach so that increased residence time will decrease the amount of parent compound available for absorption. If the ingested xenobiotic passes through the stomach unaltered, the longer time in the small intestine may increase the fraction absorbed.

Pulmonary function also changes significantly during pregnancy. Although the respiratory rate is unchanged (*14*), the tidal volume, the volume of air per breath, is increased from 487 to 678 mL (Table 11).

This means that the amount of a xenobiotic inhaled is increased during pregnancy. For example, if the work environment contains arsenic at a concentration of 0.2 mg/m^2, a nonpregnant woman will inhale 0.72 mg in an 8-hr working day. During pregnancy, that same woman will inhale 1.01 mg arsenic in the course of an 8-hr day (Table 12). Similar increases in pulmonary dose of benzene, ethylene oxide, and other airborne xenobiotics will occur during pregnancy.

It is not known if this change in pulmonary dose during pregnancy is responsible for increased maternal or fetal toxicity; however, a recent study (*15*) suggests that women are more vulnerable to silicosis than men. In this study there was a significant gender difference in the amount of time from the onset of exposure to diagnosis of silicosis. The mean duration of exposure to diagnosis was significantly ($p < 0.001$) shorter for women (20.5 ± 8.6 years), than for men (28.1 ± 10.1 years). The authors do not comment on the number of pregnancies or on the duration of work exposure during pregnancy. If these women worked during pregnancy, however, they would be inhaling greater doses of dust. Interestingly, this phenomenon of shorter latency to onset of pulmonary disease in women has also been observed in the German fire clay industry (*15*).

Table 11. Pulmonary function changes during pregnancy.[a]

Function	Nonpregnant	Pregnant	Change, %
Respiratory rate	15	16	—
Tidal volume, mL/min	487	678	+39
Minute ventilation, mL	7,270	10,340	+42
Minute O_2 uptake	201	266	+32
Vital capacity, mL	3,260	3,310	+1

[a] Data from deSwiet (*14*).

Table 12. Pulmonary dose of selected xenobiotics during pregnancy.

Xenobiotic	Nonpregnant, mg	Pregnant, mg
Arsenic, 0.2 mg/m^3	0.7	1.0
Benzene, 31 mg/m^3	116.0	162.0
Ethylene oxide, 90 mg/m^3	324.0	454.0

There are also substantial changes in blood flow to different regions of the body during pregnancy. Blood flow to the hand increases approximately sixfold during pregnancy from 3 to 18 mL/min/100 mL tissue (*16*). Blood flow to the foot doubles during gestation, increasing from 2.5 to 4 mL/min/100 mL tissue. Over this same period of gestation there are only small increases in blood flow to the forearm and leg. The increase in blood flow to the hand may have a significant impact on the amount of xenobiotic absorbed.

Distribution

During pregnancy there are changes in body weight, total body water, plasma proteins, body fat, and cardiac output that can alter the distribution of xenobiotics (*7–9*). Maternal cardiac output increases 40 to 50% by the middle of the second trimester and remains elevated throughout gestation (*16*). Maternal weight increases from 50 kg at the start of pregnancy to 63 kg at 40 weeks (*17*). Total body water increases from 25 L at the start of pregnancy to 33 L at term. Maternal extracellular fluid volume increases from 11 L to 15 L over the course of pregnancy. Plasma volume increases from 2.5 to 3.8 L over the 40 weeks of gestation.

Maternal body fat also increases about 25% during gestation (*17*). At the beginning of pregnancy the maternal body contains approximately 16.5 kg adipose tissue. At 20 weeks of gestation maternal body fat has increased to 18.5 kg, and by 30 weeks to 20 kg. This increase in body fat during pregnancy will increase the body burden of lipid-soluble xenobiotics during pregnancy, and may have an impact on the delivery of xenobiotics to the infant through the placenta and lactation.

The increase in plasma volume and total body water during pregnancy may decrease the concentration of some xenobiotics in maternal and fetal compartments. For example, if the volume of distribution in the nonpregnant woman is 5 L and if 50% of a xenobiotic (100 mg exposure) is absorbed, the initial concentration will be 10 mg/L. Suppose that during pregnancy the volume of distribution increases to 6, 7, and 8 L at 20, 30, and

40 weeks. This increase in volume of distribution will decrease the concentration of the absorbed xenobiotic to 8.3, 7.1, and 6.3 mg/L at 20, 30, and 40 weeks of gestation. Therefore, maternal sensitivity for some xenobiotics may decrease during pregnancy.

Metabolism

The altered hormonal milieu of pregnancy is associated with changes in hepatic and extrahepatic metabolism of xenobiotics (*11,12*). In addition, during gestation, metabolism by the fetus and placenta may alter maternal levels of the parent xenobiotic or its metabolites (*10*). Placental and fetal metabolism of a xenobiotic may also influence fetal or placental toxicity.

Using classical pharmacokinetics, Gillette (*10*) has evaluated the impact of fetal metabolism on maternal and fetal levels of hypothetical xenobiotics. He suggests that fetal metabolism has only a small effect on the maternal concentration of a lipid-soluble xenobiotic that is rapidly transported into the fetal compartment; however, fetal metabolism may lower the fetal concentration by half for rapidly transported lipid-soluble xenobiotics. If the xenobiotic is slowly transported to the fetus, metabolism in fetal tissues may have an even greater impact on fetal concentration. With a slower rate of transport to the fetus, metabolism reduces fetal concentrations to 20% of the concentration if fetal metabolism does not occur. Placental metabolism may also play a similar role in altering maternal and fetal concentration of some xenobiotics.

Elimination

During gestation, alterations in renal blood flow, glomerular filtration rate, hepatic blood flow, bile flow, and pulmonary function may alter maternal xenobiotic elimination (*7–9*). During pregnancy, maternal renal plasma flow increases from 500 mL/min/1.73 m^2 to approximately 700 mL/min/1.73 m^2. Glomerular filtration rate also increases during pregnancy. At the beginning of gestation, glomerular filtration rate is approximately 100 mL/min/1.73 m^2. By midgestation (20 weeks) the glomerular filtration rate has increased to approximately 150 mL/min/1.73 m^2.

Both increased renal plasma flow and glomerular filtration rate will increase the elimination rate constant for xenobiotics cleared by the kidney. If, for example, the rate constant for elimination is 0.5 min^{-1} at the beginning of gestation and increases to 0.7 min^{-1} at midgestation and 0.9 min^{-1} at term, the xenobiotic will be cleared more rapidly during pregnancy. Note that use of physiological models will directly account for this change in elimination by increased renal blood flow (*18,19*).

Consider, for example, a xenobiotic whose volume of distribution increases proportionally to maternal weight during pregnancy, and whose rate of elimination also increases from 0.10 to 0.15 min^{-1} during the first trimester. As pregnancy advances, the increased volume of distribution decreases the initial concentration of the xenobiotic in maternal plasma. The increase in elimination rate constant increases the rate at which the xenobiotic is cleared from the body. This suggests, again, that for some xenobiotics, maternal tolerance may actually increase during pregnancy; however, increased renal clearance during pregnancy may, by increasing the dose of xenobiotic delivered, increase toxicity to the maternal bladder epithelium.

Placenta

Following implantation in the primate, the placenta begins to exert control on the maternal organism. The first signal sent by the placenta, human chorionic gonadotropin (hCG), stimulates continued ovarian production of progesterone. In the absence of hCG production, or in the face of ovarian inability to respond to hCG, spontaneous abortion will occur. During implantation, therefore, the success of pregnancy depends on interactions between the ovary and placenta. Following establishment, the placenta will determine the success of the pregnancy. In other species, however, the ovary plays a more prominent role in the maintenance of pregnancy throughout its entire course (*20*).

During implantation, the placenta invades the endometrium, which formed under hormonal control of the ovary, and maternal and fetal circulatory systems are created. In primates, the maternal portion of the placenta, the lobules, are poorly defined regions separated by incomplete septa. The fetal portion of the primate placenta, cotyledons, are discrete entities. There are generally several cotyledons within each lobule. The gross and microscopic structure of the placenta is strongly dependent on the species, however, so this description of the primate placenta will not be adequate for many experimental animals (Table 13).

Exchange of proteins, amino acids, carbohydrates, fats, gases, and xenobiotics between the maternal and fetal circulatory systems occurs across the placenta. Quantitative risk assessment for teratogenicity or fetal toxicity must consider species differences in placental type and structure (Table 13). In addition, quantitative risk estimation must consider differences in placental surface area during pregnancy (Table 14), as well as differences in fetal or maternal blood flow rates through their respective circulatory units in the placenta.

Across species, for example, there are substantial differences in placental type that may explain some of the differences in response to teratogens. Rats have only a single layer of fetal cells separating the maternal circulation from the fetal circulation. In humans there are three layers separating maternal and fetal circulatory systems in the mature placenta and four layers in the first trimester placenta. This difference may, in part, account for the high false positive rate seen in the rodent (Table 3). The use of mechanism-based physiological-pharmacokinetic models, however, may allow those differences to be considered in defining human reproductive hazard and risk assessments.

Table 13. Tissue layers separating maternal and fetal circulations.

	Maternal tissue			Fetal tissue		
Placental type	Epithelium	Connective tissue	Endothelium	Trophoblast	Connective tissue	Endothelium
Epitheliochorial						
Pig	+	+	+	+	+	+
Horse	+	+	+	+	+	+
Donkey	+	+	+	+	+	+
Syndesmochorial						
Sheep	+	+	+	+	+	+
Goat	+	+	+	+	+	+
Cow	+	+	+	+	+	+
Endotheliochorial						
Cat			+	+	+	+
Dog			+	+	+	+
Ferret			+	+	+	+
Hemochorial						
Man				+	+	+
Monkey				+	+	+
Hemoendothelial						
Guinea pig						+
Rat						+

Table 14. Surface area of the human placenta during gestation.

Gestation, days	Surface area, m^2
100	1.5
120	2.5
170	4.7
190	4.9
220	7.3
240	14.0
270 (term)	15.0

Comparative surface area across species and during gestation also needs to be considered in formulating a rational risk assessment. For example, in the human the placental surface area increases from about 1.5 m^2 at 100 days gestation, to 15 m^2 at term (Table 14). It is clear that comparative transfer rates, taking into consideration number of tissue layers, distance separating circulations, and placental area must be considered in any quantitative risk assessment exploring embryonic or fetal toxicity. Those deficits represent significant information gaps in the pharmacokinetic models that can be addressed by physiological models.

Defining the effect of any chemical on the fetus, either directly or indirectly, requires elucidation of placental metabolism and transfer. At the present time, research using human fetal tissues from first or second trimester pregnancies is quite difficult for ethical, legal, and procedural factors. For that reason most research has been restricted to defining placental transfer and metabolism using term human placenta. It is hoped that with the easing of these restrictions, and with greater experience in defining placental function with term placenta, it will be possible to characterize placental function (transport and metabolism) in second and first trimester placenta. In the interim, it will be possible to approach many of these questions using animal models.

By the third trimester much of the structure of the fetus has been defined, but during this period, many of the functional characteristics of the fetus are being developed. For example, cellular communication (e.g., neuronal contacts) is being developed, as is the cell number in many organ systems. In addition, the fetus remains vulnerable to cytotoxic or disruptive processes during the third trimester. Finally, during the third trimester, issues of fetal effects from environmental exposure remains a substantial concern.

Existing evidence suggests that placental transfer from maternal to fetal circulatory system occurs for essentially every compound tested. Placental metabolism is less likely, although it has been demonstrated for selected compounds (*21–23*). When placental metabolism does occur, it may have a significant impact on fetal concentrations and fetal or placental toxicity. In addition to mediating fetal toxicity by transferring the parent compound or metabolites into the fetal circulatory system, placental toxicity by destruction of placental cells or placental functions may have similar disruptive effects on the fetus. For example, in experimental animals prenatal exposure to cadmium produces fetal death. This effect is not the result of direct fetal toxicity, but is the result of placental toxicity. For that reason, xenobiotic uptake and effect on placental function are as important as placental transport of the parent xenobiotic or metabolism and transport of metabolites to the fetus.

Physiological and Pharmacokinetic Models

Models of many types are used in all phases of biological research (*4*). The models are simplified rules or systems used to organize our view of biological structure

and function. In both an experimental and theoretical sense, models are used to define and predict the responses of complex organisms or organ systems to external forces or factors. This portion of the paper will explore pharmacokinetic models that can be used to predict human risk for adverse reproductive outcome following xenobiotic exposure.

Classical pharmacokinetic models begin with compartmental descriptions of the structure of interest. Although they are of value in many situations, these models may be limited in defining target tissue dose of a compound. In addition, classical compartmental pharmacokinetic models do not provide a direct approach to account continuously for alterations in physiology, growth, or development. As such, these compartmental models represent static images of the system at a particular time or stage of development. This should not be interpreted as suggesting that pharmacokinetic models are of little value. On the contrary, classical pharmacokinetic models can be, and have been, used to provide insight into the effects of hormonal alterations, placental function, physiological changes during pregnancy, growth, and development on xenobiotic processing and toxicity.

Physiological models represent a different approach to the formulation of a quantitative model. This approach is appealing to many biologists because physiological models retain biological, physiological, and anatomical information as discrete parameters that can be modified. The ability to modify the parameters of the model is especially appealing for biologists exploring reproductive processes with changing characteristics, including vulnerability. Physiological models are also appealing to toxicologists because they allow a direct approach for the evaluation of target tissue toxicity and metabolic cooperation between organs (e.g., maternal liver-placenta-fetal liver).

Fetal and Maternal Organisms: Three-Compartment Models

Having explored some of the physiological changes that occur during pregnancy, it is instructive to consider the effects of these changes on the amount and concentration of a xenobiotic in maternal and fetal compartments (Fig. 3). The three-compartment model used is composed of maternal central, maternal peripheral, and fetal tissues (*24,25*). Xenobiotic elimination may occur through maternal or fetal compartments; however, in these simulations we will only consider elimination through the maternal central compartment. Exchange between maternal and fetal compartments occurs across the placenta, which changes considerably during gestation (Table 14).

In these simulations, the rate of absorption (dose rate) will be determined by blood flow to the hand (Table 15). Volumes of distribution in the maternal and fetal compartments will be defined by maternal plasma volume, extravascular fluid volume, and fetal weights, respectively (Table 16). The rate of elimination from the maternal compartment will be proportional to renal plasma flow (Table 17). The rate of transfer of xenobiotics between the maternal and fetal compartment will be proportional to placental surface area (Tables 14 and 17). Note that these simulations are by no means the only

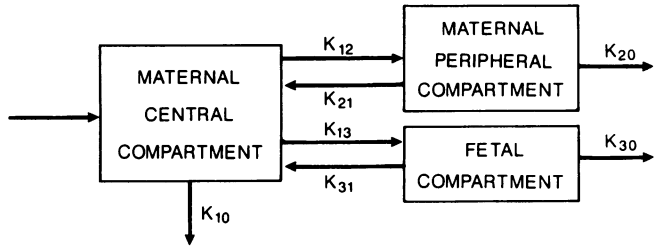

FIGURE 3. Three-compartment pharmacokinetic model during pregnancy. Parameter identification and assumptions used in this model are: V1 = MC = plasma volume = maternal central compartment; V2 = MP = extravascular volume = maternal peripheral compartment; V3 = F = 0.8 × fetal weight = fetal compartment; K_{10} = proportional to glomerular filtration rate; K_{12} = constant; K_{13} = proportional to placental surface area; K_{20} = K_{30} = 0. Transfer between compartments is by diffusion.

Table 15. Parameters used in three-compartment pharmacokinetic model of pregnancy: Absorption.

Gestation, weeks	Hand blood flow, mL/min/100 mL tissue
0	3.0
10	4.5 (1.5)[a]
20	6.0 (2.0)
30	12.0 (4.0)
40	18.0 (6.0)

[a] Values in parentheses indicate fold increase in the parameter.

Table 16. Parameters used in three-compartment pharmacokinetic model of pregnancy: Distribution.[a]

Gestation, weeks	Maternal, L		Fetal, L
	Central	Peripheral	
0	2.5	22	—
10	2.8 (1.1)	23 (1.05)	0.01
20	3.0 (1.2)	24 (1.09)	0.25 (25)
30	3.6 (1.4)	25 (1.14)	1.10 (110)
40	3.8 (1.5)	29 (2.80)	2.80 (280)

[a] Maternal central compartment is the plasma volume. Maternal peripheral compartment is the volume of extravascular water. The fetal volume of distribution is 80% of the fetal body weight. Values in parentheses represent the fold increase of the indicated parameter.

Table 17. Parameters used in three-compartment pharmacokinetic model of pregnancy: Rate constants.

Gestation, weeks	Renal plasma flow, mL/min/1.73 m^2	Placental surface area, m^2
0	500	—
10	760 (1.5)[a]	1.0
20	760 (1.5)	3.0 (3)
30	680 (1.4)	7.3 (7.3)
40	720 (1.4)	15.0 (15.0)

[a] Values in parentheses indicate the fold increase of the parameter.

ones that could have been performed. For example, it is possible to explore altered absorption in pregnancy through pulmonary function, ingestion, or transport, which is dependent on placental weight rather than surface area.

Xenobiotic Absorption Through Hand Epithelium

During pregnancy, blood flow to the hand increases approximately sixfold. If the hand is the major site of xenobiotic absorption, there will be a sixfold increase in the rate of dosing (Table 18). In this simulation, the maternal central compartment (MC) is the plasma volume, the maternal peripheral compartment (MP) is the extravascular volume, and the fetal compartment (F) is proportional to fetal body water (0.8 × fetal body weight). Elimination from the central compartment (K_{10}) occurs via the kidney and is proportional to glomerular filtration rate. Transfer from maternal central to the peripheral compartment (K_{12}) is constant throughout pregnancy. Transfer from the maternal central to fetal compartment (K_{13}) is proportional to the placental surface area. Elimination does not occur from either maternal peripheral or fetal compartments and transfer between compartments occurs by diffusion. To make the simulation somewhat more realistic, we will assume that exposure occurs only from 8:00 A.M. to 12:00 noon and again from 1:00 P.M. to 5:00 P.M., on weekdays (Table 19). Figure 4 illustrates typical daily and weekly exposure simulations for a nonpregnant woman.

In the one-day simulation, the initial concentration of the xenobiotic is zero because it is assumed that this will be the first day of exposure, on the job. Exposure then begins at 8:00 A.M. and continues until noon, when the lunch break is taken. During the lunch break the concentration in the maternal central compartment falls; little change is noted in the maternal peripheral compartment. The fetal compartment is not shown because the woman is not pregnant (Fig. 4) (Table 20).

The one-week simulation is the remainder of the week for this particular nonpregnant woman (Fig. 4). At the end of the first work day, the concentration falls over the evening, throughout the night, and begins to increase again on the morning of the second day. Ultimately, by Friday at 5:00 P.M., the maximum concentration is achieved in the maternal compartment (Fig. 4) (Table 21). Over the weekend the concentration of the xenobiotic falls in the maternal central and peripheral compartments; however, the rate of decline is very slow. At the beginning of the second week of work, therefore, the concentration in the maternal compartments is quite high and will continue to rise over succeeding weeks of exposure.

In performing these simulations in nonpregnant women and at 10, 20, 30, and 40 weeks of gestation, we have used two assumptions: first, that the increase in blood flow to the hand has no effect on xenobiotic absorption, and second, that the only significant alterations are those occurring to the maternal organism (Tables 16–18). The simulations with constant maternal exposure are shown on Figures 5 and 6 (*A,C,E,G*). The simulations with increasing maternal absorption (dose) are also shown on Figures 5 and 6 (*B,D,F,H*).

If maternal absorption does not increase during pregnancy, then the concentration in the maternal central compartment at the end of the work day falls from 2.072 prior to pregnancy to 1.042 at the end of the pregnancy (Fig. 5) (Table 20). Similarly, the concentration at the end of the work day in the maternal peripheral compartment falls from 0.116 prior to pregnancy to 0.069 at the end of pregnancy. Because of changes in the maternal organism over pregnancy with a fixed absorption, the concentration also falls in the fetal compartment at the end of the work day from 1.755 at 10 weeks to 0.620 at term. Note that during the evening the concentration increases in the maternal peripheral and fetal compartments, but that these concentrations also decrease over gestation. A similar decrease in the concentration of xenobiotic in the maternal central, peripheral, and fetal compartments will also be observed over the work week with constant maternal absorption during pregnancy (Fig. 6*A,C,E,G*) (Table 21).

If the increase in blood flow to the skin produces a similar increase in absorption, there will be an increase in the concentration in the maternal central, maternal peripheral, and fetal compartments over the course of gestation (Fig. 5*B,D,F,H*) (Table 20). For example, with increasing exposure, the concentration of xenobiotic in the maternal compartment at the end of a work day will increase from 2.072 to 6.25 at term, approximately a threefold increase compared to the pregnant constant exposure at term. The sixfold increase, con-

Table 18. Maternal and fetal concentrations: Absorption proportional to maternal hand blood flow.

Gestation, weeks	Dose, μ/hr[a]	Volumes, L			Rate constants, hr^{-1}		
		MC	MP	F	k_{10}	k_{12}	k_{13}
0	1.0/—	2.5	22	—	0.010	0.1	0.
10	1.0/1.5	2.8	23	0.01	0.015	0.1	0.01
20	1.0/2.0	3.0	24	0.25	0.015	0.1	0.03
30	1.0/4.0	3.6	25	1.0	0.014	0.1	0.07
40	1.0/6.0	3.8	29	2.8	0.014	0.1	0.15

Abbreviations: MC, maternal central compartment; MP, maternal peripheral compartment; F, fetal compartment.

[a] Exposure occurs from 8:00 A.M. to 12 noon and from 1:00 P.M. to 5:00 P.M., 5 days a week. The dose rate on the left represents unchanged absorption during pregnancy that on the right represents a dose proportional to blood flow in the hand.

Table 19. Maternal and fetal concentrations: Maternal hand blood flow.

Exposure schedule									
Day 1		Day 2		Day 3		Day 4		Day 5	
8	R[a]	8	R	8	R	8	R	8	R
12	O[b]	12	O	12	O	12	O	12	O
1	R	1	R	1	R	1	R	1	R
5	O	5	O	5	O	5	O	5	O

[a] R, Occupational exposure period.
[b] O, Time at which occupational exposure stops.

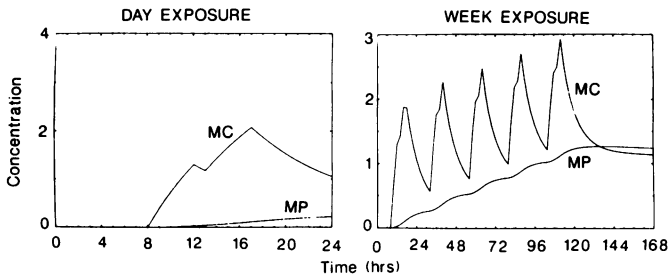

FIGURE 4. Simulations of maternal central (MC) and maternal peripheral (MP) concentrations of a xenobiotic absorbed through hand epithelium in an occupational setting for a nonpregnant woman. Exposure and absorption only occurs between 8:00 A.M. to 12 noon and 1:00 P.M. to 5:00 P.M. Left: 1-day exposure. Right: 5-day exposure.

sistent with the increase in blood flow to the skin, will be reflected in a similar increase in the concentrations of the maternal peripheral and fetal compartments.

In the one-day simulations, the maximum concentration in the maternal compartment will occur at 5:00 P.M., the end of the exposure period (Table 20) (Fig. 4 and 5). With fixed exposure over the course of gestation, the xenobiotic concentration in the maternal central compartment will decrease from 2.072 to 1.042 μ/L from the nonpregnant state until term, 40 weeks gestation, (Fig. 5). Maternal peripheral concentrations will be about 10% of those in the central compartment, whereas fetal concentrations will be quite close to those in the maternal central compartment at 10 weeks. Over the course of pregnancy, however, the fetal concentration will decline more than the maternal concentrations. The greatest concentrations in the fetal compartment will therefore be achieved early in pregnancy. As expected, increasing exposure increases the xenobiotic concentration in the maternal central, maternal peripheral, and fetal compartments; however, the increased concentration is less than the sixfold increase in blood flow. This is due to the parallel increase in volumes of distribution. Similar changes in xenobiotic concentration are noted at the end of the work week (Table 21).

In these simulations, if absorption is not altered during pregnancy, the maternal organism is exposed to the highest concentrations of the xenobiotic in the nonpregnant state, and fetal concentrations are highest during the first trimester, falling as pregnancy advances. This suggests that for some compounds, maternal toxicity may actually decrease during pregnancy. If absorption increases during pregnancy, then the maternal central, peripheral, and fetal concentrations will increase during pregnancy. With increasing maternal absorption, the likelihood of maternal toxicity will increase during pregnancy as will the risk for fetal toxicity.

Conclusions and Recommendations

This is an exciting era for research in risk assessment in reproductive toxicology. A range of *in vitro*, *in vivo*, and theoretical models are being explored that offer promise for understanding normal and pathological reproduction and development. These models also offer toxicologists the opportunity to define common links between species for the formulation of risk assessments with well-characterized uncertainty.

The development of risk assessment in reproductive and developmental toxicology across species, however, will not come without research initiatives in several areas. The areas that are most likely to be productive include: establishment of a data base in reproductive pharmacology, toxicology, and physiology; development of pharmacokinetic and physiological models; validation of the physiological and pharmacokinetic models with

Table 20. Concentration at the end of a single work day: Absorption proportional to blood flow to the hand epithelium.

Gestation, weeks	Fixed exposure			Increasing exposure		
	MC	MP	F	MC	MP	F
0	2.072	0.116 (0.221)[a]	— (—)	— (—)	— (—)	— (—)
10	1.814	0.109 (0.205)	1.755 (1.755)	2.721	0.164 (0.307)	2.633 (2.633)
20	1.632	0.101 (0.189)	1.210 (1.329)	3.263	0.202 (0.378)	2.420 (2.659)
30	1.262	0.090 (0.166)	0.788 (0.912)	5.049	0.361 (0.664)	3.151 (3.650)
40	1.042	0.069 (0.125)	0.620 (0.717)	6.250	0.416 (0.750)	3.720 (4.300)

[a] Numbers in parentheses represent the maximum concentration reached in the maternal peripheral or fetal compartments.

Table 21. Concentration at the end of a work week: Absorption proportional to blood flow to hand epithelium.

Gestation, weeks	Fixed exposure			Increasing exposure		
	MC	MP	F	MC	MP	F
0	2.926	1.123 (1.264)[a]	— (—)	— (—)	— (—)	— (—)
10	2.535	1.005 (1.120)	2.473 (2.473)	3.802	1.507 (1.680)	3.710 (3.710)
20	2.336	0.949 (1.059)	1.954 (2.055)	4.672	1.897 (2.119)	3.910 (4.110)
30	1.970	0.875 (0.980)	1.586 (1.678)	7.881	3.501 (3.919)	6.346 (6.712)
40	1.688	0.721 (0.820)	1.335 (1.403)	10.126	4.325 (4.920)	8.011 (8.418)

[a] Numbers in parentheses represent the maximum concentration reached in the maternal peripheral or fetal compartments.

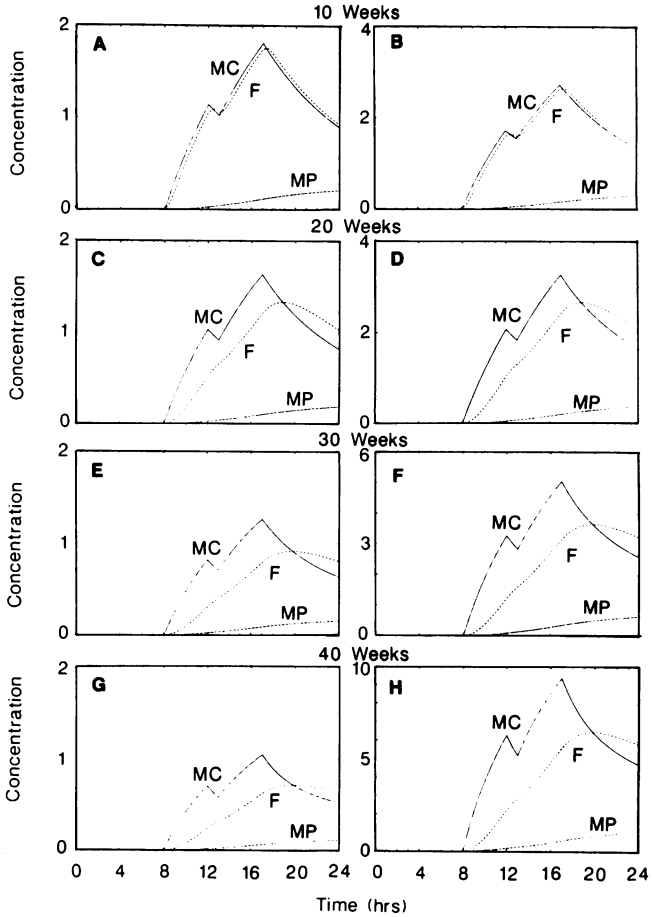

FIGURE 5. Simulations of maternal central (MC), maternal peripheral (MP), and fetal (F) concentrations of a xenobiotic absorbed through hand epithelium during the first day of occupational exposure at 10 (*A,B*), 20 (*C,D*), 30 (*E,F*), and 40 (*G,H*) weeks of gestation. Simulations with constant absorption through the hand during pregnancy are illustrated by panels on the left. Simulation with increasing absorption through the hand during pregnancy are illustrated on the right.

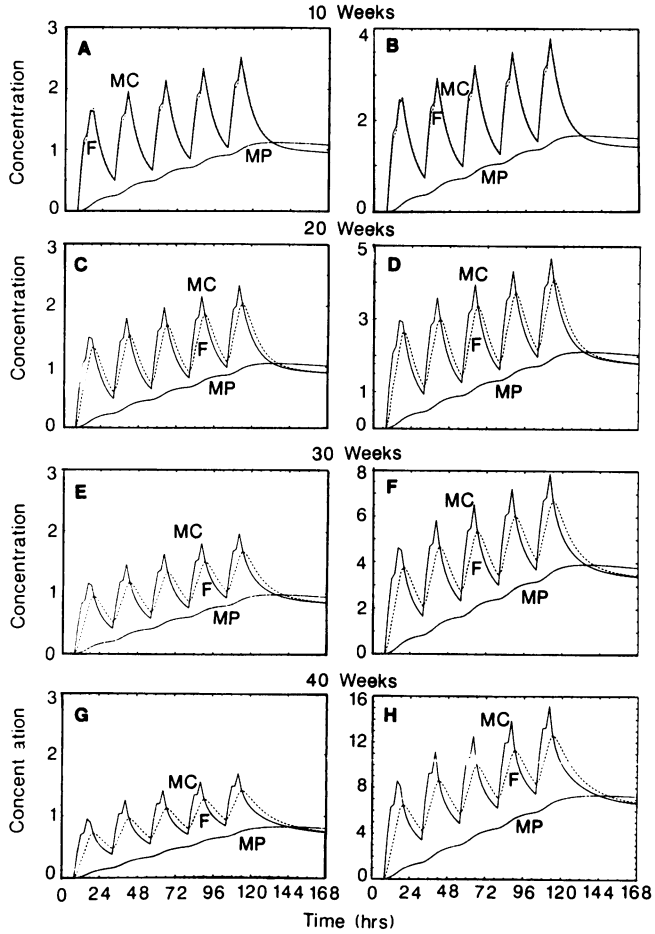

FIGURE 6. Simulations of maternal central (MC), maternal peripheral (MP), and fetal concentrations (F) of a xenobiotic absorbed through the hand epithelium during the first week of occupational exposure at 10 (*A,B*), 20 (*C,D*), 30 (*E,F*), and 40 (*G,H*) weeks of gestation. Simulations with constant absorption through the hand during pregnancy are illustrated by panels on the left. Simulations with increasing absorption through the hand during pregnancy are illustrated on the right.

concurrent experimentation using *in vivo* and *in vitro* systems; and development of expert systems for consistent prediction of human reproductive risk from animal data.

At the present time, the most compelling need is the development of data bases of physiological, pharmacokinetic, and metabolic parameters within and across species during gestation and development. Once data are available, it will be possible to begin testing mechanism-based physiological and pharmacokinetic models and expert systems for quantitative risk assessment. The data base, alone and together with the physiological and pharmacokinetic models, will also suggest *in vivo* and *in vitro* experiments to validate the risk estimates and reduce their uncertainty. Finally, the data base will be a reusable and continuously growing scientific resource.

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