SECTION I

METABOLISM AND KINETICS OF TOXIC CHEMICALS
I. FUNDAMENTAL CONCEPTS

by
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In the safety evaluation of chemicals, the basic reasons for undertaking metabolism and toxicokinetic studies are to elucidate the mechanisms of toxicity of the chemicals in question and to facilitate the extrapolation of experimental animal toxicity data to humans. Chemical toxicity arises from reactions between the ingested toxic chemical, or one or more of its metabolites with chemical constituents of the body. For example, an ingested chemical A may be metabolized into a "reactive intermediate" B, which can then undergo further metabolism by interaction with a body constituent, such as glutathione, to give metabolite C, which is then excreted in the urine or bile. Alternatively, "reactive intermediate" B may undergo further metabolism to a "proximate carcinogen" D, which may alkylate DNA, resulting in mutations and possibly carcinogenesis. The toxicity of the chemical will depend on the concentration of the "reactive intermediate" in the target tissue which in turn will depend on the rates of the alternative reactions B⟶C, and B⟶D. Reaction B⟶C would generally be a "detoxication" reaction, whereas the reaction B⟶D is a "metabolic activation".

A knowledge of the changes which the ingested chemical may undergo in the body enables us to understand the molecular mechanisms of toxicity. For example, the formation of the reactive intermediate epoxides of the polycyclic hydrocarbons leads to their interaction with glutathione (detoxication) or with DNA (activation) or results in their further metabolism to the less reactive phenols (detoxication). A knowledge of metabolism has also recently been shown to provide information on the likely integrity of the body's defence mechanisms against chemicals. Generally, these mechanisms act to protect the animal organism against the toxic effects of chemicals by detoxicating them and to protect against the toxicity of excess tissue oxygen (1,2). Interference with these enzymic detoxication mechanisms by highly toxic chemicals may result in toxicity not only from the toxic chemicals *per se* but also from autoxidative mechanisms resulting from damage of the body's chemical defence system. For

example, halogenated hydrocarbons may form ligand complexes with the cytochrome P-450 of the mixed-function oxidase system, leading to autoxidation and lipid peroxidation (2,3).

A study of the metabolism and toxicokinetics of chemicals is essential in animal toxicology studies conducted in the safety evaluation of chemicals for the following reasons:

- to determine the rate of absorption, distribution, metabolism and elimination of the chemical in the particular animal species under study to understand the mechanism(s) of toxicity and to identify the target organ(s) for chemical toxicity;

- to enable the selection of the most appropriate animal model for toxicity studies by comparison with the chemobiokinetics and metabolism of the chemical in humans;

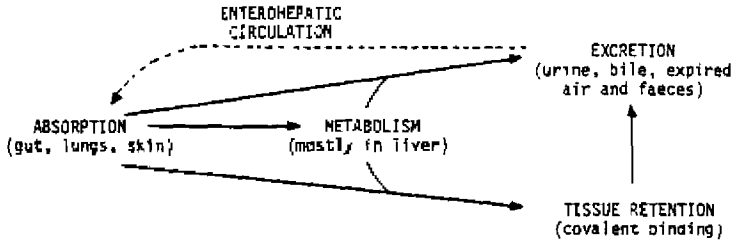
- to enable calculation of the kinetically equivalent human dosage for administration to the animal species in the toxicity studies; and

- to facilitate the quantitative interpretation of animal toxicity data relevant to human safety.

Detoxication and Activation

When toxic, particularly polar, nonlipophilic, chemicals are ingested, they may be rapidly excreted, or, if they are lipophilic, they may undergo metabolism by which they are converted into more polar, water-soluble compounds, which are more readily excreted (Fig. 1). The original chemical or any of its metabolites may then be distributed into the tissues or excreted from the body. In this way lipophilic chemicals are made more polar and are eventually eliminated from the body in the urine or bile. Many compounds which are excreted into the bile may undergo further metabolism in the gastrointestinal tract, to be reabsorbed and then excreted once again in either the bile or the urine - a process known as enterohepatic circulation.

Fig. 1. Disposition of toxic chemicals in the animal body



The various enzymic reactions which may occur during the metabolism of toxic chemicals have been classified into biotransformation reactions (Phase I reactions) and conjugation reactions (Phase II reactions). These biotransformation and conjugation reactions may lead either to the detoxication of the chemical and the excretion of its metabolites or to activation of the chemical into reactive intermediates which may then interact with glutathione, tissue proteins, RNA or DNA to give rise to various toxic reactions. As an example of detoxication and of Phase I and Phase II reactions, toluene may undergo oxidation to benzoic acid, then conjugation with glycine to yield hippurate, which is finally excreted in the urine. The major types of biotransformation reactions (Table 1) consist of: oxidations, reductions and hydrolyses catalyzed by the enzymes of the endoplasmic reticulum (microsomal enzymes) or by nonmicrosomal enzymes of the cell cytosol, mitochondria or the blood plasma; and reductions and hydrolyses (but not oxidations) catalyzed by enzymes of the microflora of the gastrointestinal tract. The principal sites of metabolism by mammalian enzymes are the liver and the gastrointestinal tract, with lesser activity present in the lungs, kidneys and skin.

Table 1. Classification of the metabolic transformation of drugs

Chemical reaction	Microsomal drug-metabolizing enzymes	Nonmicrosomal mammalian enzymes	Enzymes of the intestinal microflora
Oxidation (oxygenation)	Aromatic hydroxylation	Alcohol oxidation (cytoplasm)	
	Acyclic hydroxylation	Aldehyde oxidation (cytoplasm)	
	Alicyclic hydroxylation	Alicyclic aromatization (mitochondria)	
	Epoxidation		
	N-Oxidation		
	S-Oxidation		
	Desulfuration		
	Dealkylation		
	Deamination	Deamination (mitochondria and blood plasma)	
Reduction	Nitro reduction	Reduction of sulfoxides and N-oxides (cytoplasm)	Reduction of N-oxides
	Azo reduction	Reduction of disulfides	Azo reduction
	Dehalogenation		Dehydroxylation
Hydrolysis	Hydrolysis of esters	Hydrolysis of esters and amides (blood plasma)	Hydrolysis of esters
		Hydrolytic ring scission	Hydrolytic ring scission
		Dehalogenation (cytoplasm)	

SECTION I

The endoplasmic reticulum is an intracellular membranous system, well developed in most epithelial cells, associated with a number of functions, in particular the synthesis of intracellular and extracellular glycoproteins, substances essential for the function of the cell itself and also for the whole living organism (2). Examples of liver microsomal mixed-function oxidations include aromatic and aliphatic hydroxylation, epoxidation, N-oxidation, S-oxidation, dealkylation and deamination. Examples of nonmicrosomal oxidation include alcohol dehydrogenase, aldehyde dehydrogenase and monoamine and diamine oxidase. The various enzymic reductions and hydrolyses include nitro-reductase, azo-reductase, reductive dehalogenation, aldehyde reduction and hydrolysis of esters and amides. In the conjugation reactions, the toxic chemical or its metabolites are enzymically coupled with various endogenous molecules to make them more hydrophilic, more polar and more readily excreted from the animal organism. These endogenous small molecules may be sugars (glucose and glucuronic acid), acids (sulfuric, acetic), amino acids and peptides (glycine, glutamine and glutathione), alkyl groups (methyl), etc.

Xylenes, widely used as solvents, may undergo oxidation of a methyl group to yield toluic acids which are then excreted in the urine as glucuronide or sulfate conjugates. Alternatively, the xylenes or the toluic acids may undergo ring hydroxylation to give the xylenols or hydroxytoluic acids, respectively, which are then conjugated with glucuronic acid and sulfate prior to excretion in the urine. Aniline may undergo direct conjugation of the amino group with sulfate or glucuronic acid to give the sulfamate and the N-glucuronide, respectively, or undergo ring hydroxylation to give the isomeric orthometa- and para-aminophenols excreted as their glucuronide and sulfate conjugates or N-hydroxylation to give phenylhydroxylamine which is also excreted conjugated. A more extensive pattern of metabolism is seen with the petrochemical benzene which may undergo ring hydroxylation to give phenol and then further hydroxylation to yield catechol, quinol and hydroxyquinol. The catechol may undergo ring scission to yield muconic acid and evantually CO_2, in addition, benzene may also react with glutathione to yield, eventually, a small amount of phenylmercapturic acid which is excreted in the urine. Styrene, a widely used chemical in the plastics industry, contains an unsaturated bond in

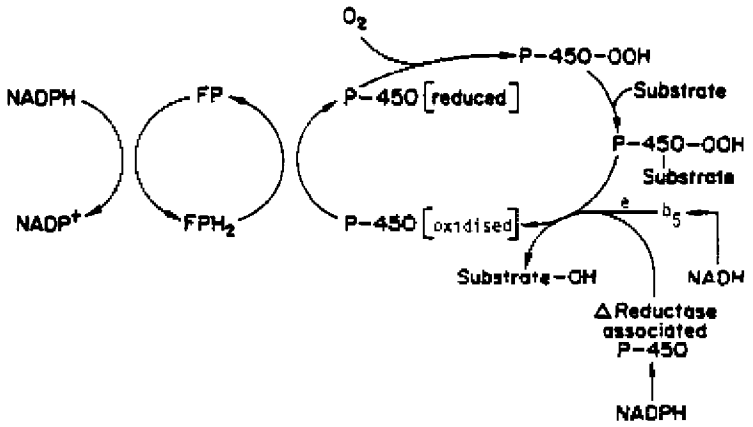
the aliphatic side-chain, and this bond undergoes oxidative metabolism to form styrene epoxide: this epoxide is subsequently hydrated by epoxide hydrase to give phenylglycol which may undergo further oxidation to yield mandelic acid or undergo oxidative decarboxylation to benzoic acid.

Microsomal Mixed-Function Oxidases

The mixed-function oxidase reactions are catalyzed by a microsomal enzymic system consisting of cytochrome P-450 (so named because of spectral absorption at 450 nm), which is the terminal oxygen transferase, coupled to cytochrome P-450 reductase (containing both flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)) and linked to a source of electrons from nicotinamide adenine dinucleotide phosphate (NADPH). This mixed-function oxidase system, in addition to catalyzing the oxidative metabolism of environmental chemicals including drugs, pesticides, food additives and industrial intermediates, also oxygenates sterols, steroids and fatty acids and is responsible for the biosynthesis and deactivation of steroid hormones, prostaglandins, prostacyclins and thromboxanes (2,4). Not surprisingly, therefore, interactions of toxic chemicals with this ubiquitous and important enzyme system can have far-reaching effects on intermediary metabolism and on the whole economy of the cell and the animal organism. The cytochrome P-450 system is now believed to also have a role in the detoxication of oxygen which can become one of the most cytotoxic chemicals normally found in living tissues (5). Cytochrome P-450 is thought to function by converting molecular oxygen into superoxy anion which is then converted into peroxide by superoxide dismutase and then to water by catalase. This detoxication of oxygen prevents excess molecular oxygen in the cell from being activated by flavoproteins into singlet oxygen, hydroxyl radicals and other highly reactive and toxic species (2).

Cytochrome P-450 catalyzes the insertion of an atom of oxygen from O_2 into the chemical substrate RH while the other atom of oxygen associates with two protons and the gain of two electrons to yield a molecule of water: hence the classification of this system as a "mixed-function oxidase" (Fig. 2) (6,7). Recent studies of this membrane-bound enzyme system have enabled the enzyme proteins to be solubilized, separated and purified. They

Fig. 2. Schematic diagram of microsomal mixed-function oxidation



have thus revealed that a number of cytochromes P-450 and cytochrome P-450 reductases exist, and that lipid, especially phosphatidylcholine, is essential for this enzymic activity (8). These multiple cytochromes P-450 are generally regarded as isoenzymes or enzyme variants, but another form of cytochrome P-450, found in greater abundance in embryonic and neonatal tissues, is also the form of this enzyme found in neoplastic tissue. This form of the enzyme is known as cytochrome P-448 (because its reduced CO-ligand spectrum has a maximum at 448 nm). It is normally present in the high-spin form (5-ligand complex) whereas cytochrome P-450 is normally present in the low-spin form (6-ligand complex). The sixth ligand of cytochrome P-450 is occupied by the hydroxyl group of an amino acid (or by the nitrogen of a histidine moiety) of the apoenzyme of cytochrome P-450 and can be displaced by nitrogenous bases, thiols and various other toxic chemicals which can form stable complexes with cytochrome P-450 (Fig. 3). In the normal mixed-function oxidase activity of cytochrome P-450, the enzyme changes from a low-spin state (6 ligands) to a high-spin state (5 ligands with the apoenzyme ligand broken), with a change in the

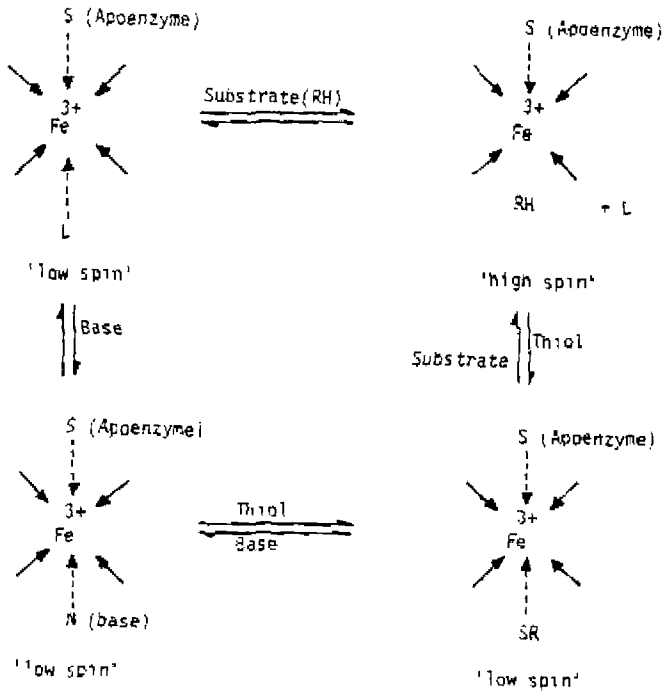
conformation of the enzyme prior to activation of the oxygen and its transfer into the organic substrate. Hence, if a stable ligand complex of cytochrome P-450 is formed by interaction with toxic chemicals, the enzyme is generally unable to carry out its normal function of mixed-function oxidation and instead appears to catalyze cytotoxic autoxidation processes (2). Many environmental chemicals are now known to undergo metabolism by cytochrome P-450 with the formation of carbenes and possibly other highly reactive intermediates which then form stable ligand complexes with cytochrome P-450, thus inhibiting its normal enzymic activity. Several halogenated hydrocarbons, such as halothane and the Freons, are known to undergo reductive dehalogenation with the formation of carbenes which then form ligand complexes with cytochrome P-450. This action is often an alternative to oxidative metabolism by cytochrome P-450. Whereas reductive metabolism in the case of halothane leads to the formation of a stable carbene complex of cytochrome P-450, oxidative metabolism in the presence of NADPH results in hydroxylation of the halogenohydrocarbon with the eventual formation of trifluoroacetic acid (3).

Evidence is growing that this interaction of toxic chemicals with cytochrome P-450, with the formation of stable complexes, the loss of normal detoxicating mixed-function oxidase activity and the increase in autoxidation and lipid peroxidation may be one of the major fundamental mechanisms of chemical toxicity.

Toxicokinetics

A knowledge of the rates of formation, reaction with tissue components and excretion of various metabolites is essential for a full understanding of the disposition and elimination of the toxic chemical from the body, and of the mechanism and extent of its toxicity. Fig. 4 shows a typical plasma concentration/time plot of an ingested environmental chemical. The blood/plasma concentration of the chemical usually gives a good correlation with the concentration of the chemical in the body's tissues and generally parallels the rates of change of concentration of the chemical in the tissues. The slope of the log concentration/time curve is not linear and consists in this case of three different slopes or rates of decay (P-1, P-2 and P-3) which correspond with different

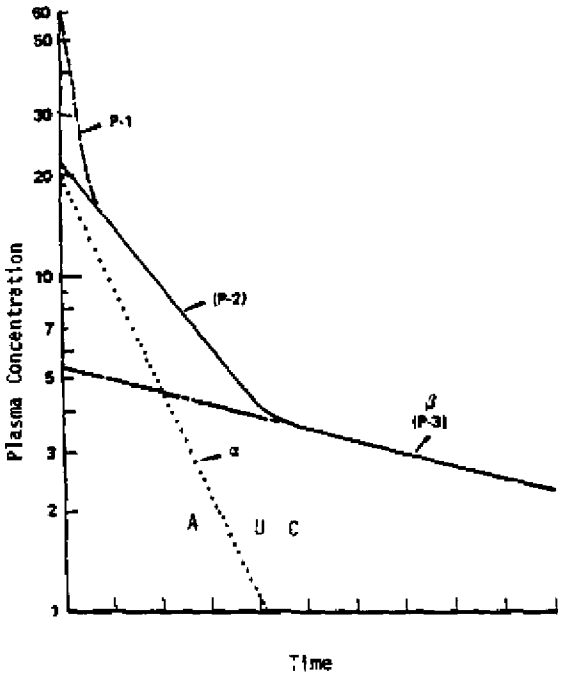
Fig. 3 Ligand complexes of cytochrome P-450



processes of disposition of the chemical. From these slopes may be calculated the plasma half-lives ($t_{\frac{1}{2}}\alpha$ and $t_{\frac{1}{2}}\beta$) which are indices of the period of time during which the chemical remains in the body. The area under the slope of this curve (AUC) is an index of absorption and persistence in the body of chemicals which have entered through the mouth, nose or skin and of the persistence of those chemicals administered parenterally. From the slope of the curve the elimination constant (k_{el}) may be calculated, and from the AUC the volume of distribution

(V_d), and the clearance (Cl)[a] may be calculated. These quantities ($t_{\frac{1}{2}}$, AUC, V_d, Cl) are the most widely used pharmacokinetic parameters and enable the rate of absorption, distribution and excretion of the chemical and its various metabolites to be quantified.

Fig. 4. Plasma concentration time/curve for a chemical administered intravenously



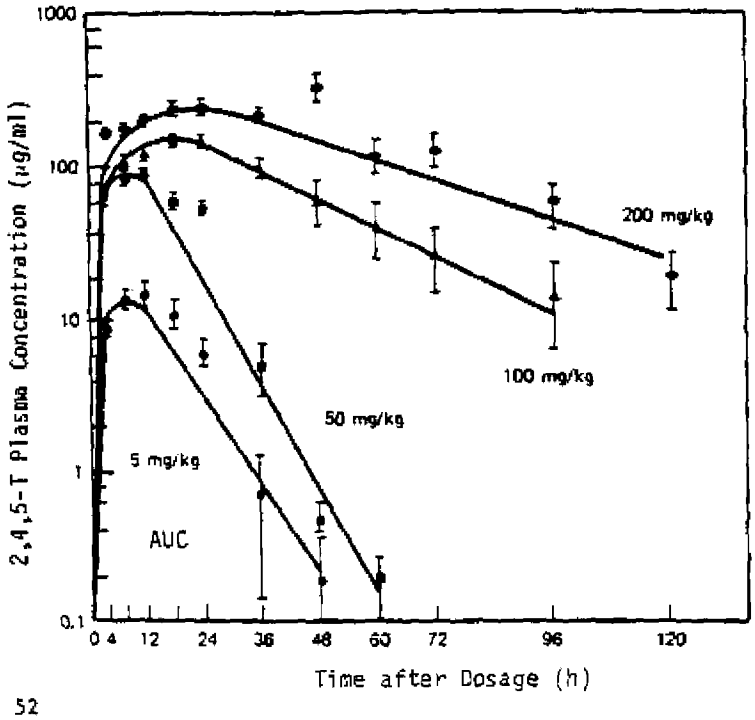
[a] $k_{el} = \frac{0.693}{t_{\frac{1}{2}}}$ where the chemical is eliminated by first order kinetics,

$V_d = \frac{D_{i.v.}}{AUC \cdot k_{el}}$ where $D_{i.v.}$ is the intravenous dose

$Cl = V_d \cdot k_{el} = \frac{D_{i.v.}}{AUC}$

The area under the curve is, of course, much affected by the dose of the chemical ingested. For example, a 10-fold increase in the dose of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in the rat increases the plasma maximum concentration and the area under the curve approximately the same extent (Fig. 5). Although a 10-fold increase of the dose from 5 mg/kg to 50 mg/kg does not change the slope of elimination, further increases of dose to 100 and 200 mg/kg do result in a marked change, indicating that at these higher doses the rate of metabolism, distribution or excretion has become markedly slower. This change suggests that some process has become saturated; for example, tissues have become saturated with the 2,4,5-T, a metabolic process has become saturated or possibly an enzyme poisoned by the excessively high dosage, thus resulting in prolonged retention of the chemical.

Fig. 5. Plasma concentration/time curves for different oral doses of 2,4,5-trichlorophenoxyacetic acid administered to rat (from ref. 9)



The plasma concentration does not always parallel the concentration in the various body tissues as when a specific tissue affinity is present for a given chemical. For example, paraquat has a high affinity for the lung. The paraquat concentration in the lung increases during the first two hours whereas the concentration in the blood plasma falls rapidly during this time. From the period two hours onwards, the $t_{\frac{1}{2}}$ for paraquat in the lung is approximately 20 hours, whereas in the blood plasma $t_{\frac{1}{2}}\alpha$ is about 1 hour and $t_{\frac{1}{2}}\beta$ about 20 hours, the same as that in the lung (10).

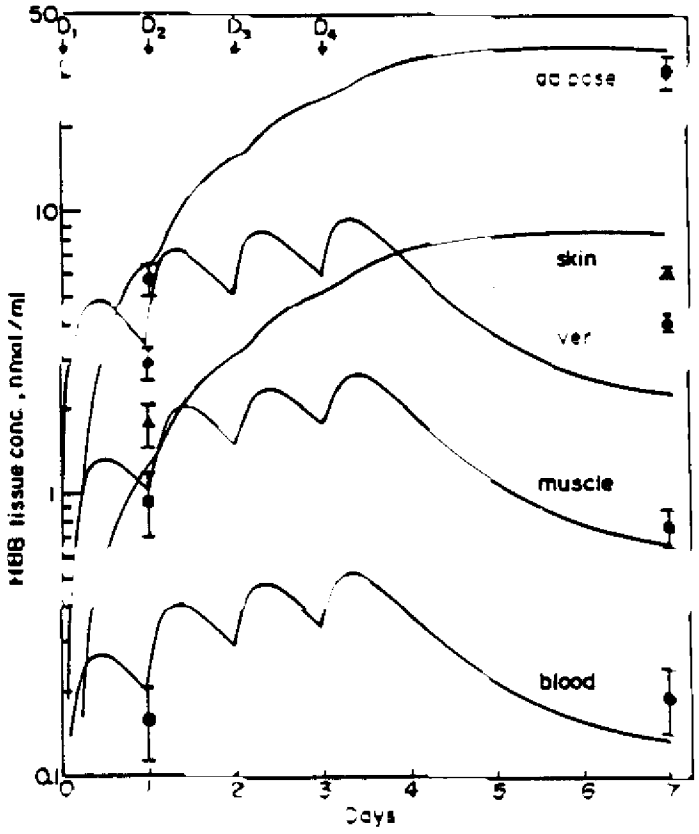
With highly lipophilic compounds such as hexabromobiphenyl (which has a high affinity for lipid), the concentration in adipose tissue and in skin (which contains much adipose tissue) may increase while the concentration in other tissues (blood plasma, liver and muscle) falls. On repeated dosage (Fig. 6) the concentration in blood, liver and muscle may show a daily variation and then decline after cessation of repeated dosage while the concentration in adipose tissue and skin continues to increase and then plateaus after termination of dosage (11).

By using an animal model such as the rat, experimental distribution studies may be carried out, and from a calculation of the kinetic parameters, computer programmes can be designed to predict the plasma and tissue concentrations on repeated dosage at various dose levels (11). Computer programmes such as these, validated for humans, can be valuable in the accurate prediction of blood and tissue concentrations of human individuals exposed to known environmental concentrations of a chemical. In addition, a knowledge of the mechanisms of toxicity, the limits of detoxication and the threshold values may permit accurate prediction of the environmental concentrations of the chemical considered safe for human subjects.

Finally, the determination of the toxicokinetic parameters ($t_{\frac{1}{2}}$, AUC, V_d, Cl) and the pattern of metabolism of the chemical at several different dose levels, particularly those doses used for the animal toxicity studies is highly desirable because the toxicity of the chemical may change with the dose, becoming greater at higher doses saturating the pathways of detoxication and excretion and leading to progressive accumulation and toxicity. In the absence of this information, the evaluation of chemical toxicity,

especially at high dosage, is difficult, and assessment of the hazard of human exposure may become erroneous.

Fig. 6. Distribution with time of hexabromobiphenyl in tissues of rat after repeated oral dosing



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