Answers to questions

Chapter 1

A1. (d) Potentiation.

The compound B is not toxic, yet when combined with A the toxicity is increased. Thus, compound B potentiates the toxicity of A. This is distinct from synergism where both compounds are toxic but the toxicity of the combination is more than the sum of the individual toxicities.

A2. (e) All of the above.

A properly designed and carefully executed and observed acute toxicity study may give information on all of these parameters. (The therapeutic index may be determined provided the ED_{50} is known.) The LD_{50} is now rarely required as an end in itself except for specific situations such as the design of pesticides. However, with a novel compound where the toxicity and lethality are unknown, doses that prove to be lethal may be administered to animals in preliminary toxicity studies. Consequently, an LD_{50} could be calculated from such data.

A3. (c) LD_{50}/ED_{50}.

Therefore the bigger the therapeutic index the safer the drug. A better and more discriminating definition would be TD_{50}/ED_{50} in which the toxicity rather than the lethality is used for the numerator.

SHORT ANSWER QUESTIONS

A4. (a) TD_{50} is the dose of a compound that is toxic to 50 per cent of the population exposed to that compound. The value is determined from the dosage–response relationship by interpolation (see Figure 1.5).

(b) Dosage (dose)–response relationship is the mathematical relationship between the dosage (dose) of a compound and the particular response measured. The response may be quantal (e.g. 'all-or-none' such as lethality) or graded (e.g. inhibition of an enzyme). The relationship is typically a sigmoid curve. This reflects the fact that there are doses that have no effect and those that have a maximal effect (see Figure 1.4).

(c) Therapeutic index is an index of relative toxicity for a drug. Calculated as the LD_{50} or TD_{50} divided by the ED_{50}. Thus the greater the number, the less toxic the drug is relative to the pharmacologically effective dose. This is reflected in Figure 1.5.

(d) NOEL (The **N**o **O**bserved **E**ffect **L**evel) is the dose or exposure level of a chemical which has no demonstrable effect on a biological system. (NOAEL: No Observed Adverse Effect Level.) The value may be derived from the dosage–response relationship. (See Figure 1.7.)

A5. Selective toxicity means that certain organisms are susceptible to a toxicant and others are not. For example, insects are susceptible to the toxicity of DDT and malathion whereas mammals are generally not. Bacteria are susceptible to drugs such as penicillin whilst humans are not. This selectivity in the toxic effects is therefore exploited in drugs used to fight infections, cancer and to remove pests such as insects. The basis of the selectivity may be metabolic or structural. For example the malathion is metabolized differently in insects to mammals and the bacterial cell wall is structurally different from the

mammalian cell membrane and so is a target for penicillin.

A6. (a) The ED_{50} is the dose of a compound that causes an effect in 50 per cent of the organisms or a 50 per cent response. This is usually a pharmacological, as distinguished from a toxicological, effect. The ED_{50} may be determined in a population of organisms or in an *in vitro* system. It may be a quantal ('all-or-none') response such as presence or absence of a pharmacological change or a graded response observed in the effect (see Figure 1.5).

(b) The ADI is defined as the 'acceptable daily intake'. This is usually applied to food additives or contaminants (such as pesticides). It is the calculated amount of a substance to which humans can be permitted to be exposed safely. It is calculated from the No Observed Adverse Effect Level (NOAEL) thus:

$$\text{ADI} = \frac{\text{NOAEL mg kg}^{-1}\,\text{day}^{-1}}{100}$$

The value of 100 is the safety factor that is applied. This takes into account the possible differences in susceptibility between the species used to determine the NOAEL and humans. A higher safety factor may be applied in some cases.

(c) The margin of safety is similar to the therapeutic index but more critical. Thus it is an indication of the difference between the dose or concentration of a drug required for the desired pharmacological effect and the dose associated with a toxic effect. It is calculated as follows:

$$\text{margin of safety} = \frac{TD_1}{ED_{99}} \text{ or } \frac{LD_1}{ED_{99}}$$

where TD_1 and LD_1 are the doses that are toxic or lethal for 1 per cent of the population exposed and the ED_{99} is the dose that is effective in 99 per cent of the population. (See Figure 1.5.)

The margin of safety is more critical than the therapeutic index because it takes into account possible overlap in the dose–response curves for pharmacological and toxicological effects.

Chapter 2

A1. (c) Potential to bioaccumulate.

The larger the partition coefficient the greater is the lipophilicity and this correlates with the bioaccumulation of the compound in fat tissue.

A2. (c) Weak organic acids

In the stomach the pH is around 2 and at this pH weak acids will be non-ionized. Therefore passive absorption of the non-ionized acid will occur in the stomach.

A3. (b) Sometimes larger than the total body volume.

When a drug is bound to tissue components or sequestered in a tissue such as adipose tissue, the plasma level may be very low. Therefore the calculation of volume of distribution (V_D = dose/plasma level) yields a value which may be higher than the total body water. The volume of distribution may be equal to the total body water but this is not always the case.

A4. (f) The total body clearance. Although some of the other factors may have an effect on half-life, by definition the total body clearance is the major determining factor as this includes metabolism and excretion.

A5. (b) The drug is mostly metabolized by the gastrointestinal tract and/or liver before reaching the systemic circulation.

The 'first-pass effect' is where a drug is removed by metabolism in the organ(s)/tissues through which it passes during absorption and before reaching the systemic circulation. This

is commonly the gastrointestinal tract and liver, but could also be the lungs or skin.

A6. (b) False.

Non-ionized compounds are more readily absorbed by passive diffusion as they more readily pass through the lipid bilayer parts of biological membranes.

A7. (b) False.

The binding of drugs to plasma proteins only rarely involves covalent binding. Usually ionic, hydrogen, hydrophobic or van der Waals' forces are involved.

A8. C.

Both lipophilicity and resistance to metabolism will favour accumulation of chemicals in biological systems. The former will result in sequestration in adipose tissue, the latter will decrease removal of the chemical by metabolism to polar, hydrophilic metabolites and loss by excretion.

A9. C.

Chemicals with a long half-life are more likely to accumulate on repeated dosing especially if the dosing interval is shorter than the half-life.

SHORT ANSWER QUESTIONS

A10. (a) The volume of distribution (V_D) is the volume of body fluid in which a chemical is apparently distributed after administration to an animal. It is calculated from either the dose and plasma (blood) concentration at a single time point or from the dose, area under the curve (AUC) and elimination rate constant (k_{el}):

$$V_D = \frac{\text{dose (mg)}}{\text{plasma concentration (mg L}^{-1})}$$

The units are therefore in litres.

The volume of distribution does not necessarily equal a compartment and so may have a value higher than the total body water (40 litres for a human). This occurs if the plasma level is low, as when a drug is sequestered such as in adipose tissue. V_D is therefore known as apparent volume of distribution. The volume of distribution should not be calculated after the drug is administered orally as there may be incomplete absorption and/or first-pass metabolism.

(b) Drugs normally bind to plasma proteins non-covalently and in one of four ways:

(i) by ionic bonds in which there is bonding between charged groups or atoms and opposite charges on the protein.
(ii) by hydrogen bonds where a hydrogen atom attached to an electronegative atom (e.g. oxygen) is shared with another electronegative atom.
(iii) by hydrophobic interactions in which two non-polar, hydrophilic groups associate and mutually repel water.
(iv) by van der Waals' forces: these are weak forces acting between the nucleus of one atom and the electrons of another.

There may be several molecules of drug bound to one protein molecule and strength may vary depending on the type of binding. However, binding is normally reversible. The protein commonly involved in binding is albumin. Binding to plasma proteins may increase the half-life and limit the distribution and metabolism of a drug. Drugs bound to plasma proteins may be displaced by other drugs, leading to a large rise in the free concentration in the plasma. Similarly increasing the dose of a drug which is bound extensively to plasma proteins may saturate the binding sites and lead to a sudden increase in plasma level.

(c) The first-pass effect is the extensive metabolism of a drug either by the organ of absorption or the liver following oral administration. This may lead to the situation where very little of the parent drug is distributed around the body. Thus after oral absorption a drug may

be metabolized by the gastrointestinal tract and/or the liver before reaching the systemic circulation. Therefore if the parent drug is active, little may reach the target site. If the metabolism is saturable, however, increasing the dose may dramatically increase the systemic exposure. The lungs and skin, the other organs of absorption, may also carry out first-pass metabolism. Figure 2.10 shows the effect of the first-pass metabolism of a compound after oral and intravenous administration.

(d) Fick's law of diffusion describes the relationship between the rate of diffusion of a chemical across a membrane and certain characteristics of the membrane. In the context of toxicology and drug disposition it relates to passage across a cell membrane by simple diffusion. Thus:

$$\text{rate of diffusion} = KA(C_2 - C_1)$$

where K is the diffusion coefficient, A is the surface area, C_2 is the concentration of compound outside the membrane, C_1 is the concentration of compound on the inside of the membrane. The diffusion coefficient will incorporate physico-chemical characteristics of the chemical such as lipophilicity, size, shape, etc.

A11. (a) The pH partition theory states that only non-ionized lipid soluble compounds will be absorbed by passive diffusion down a concentration gradient. For absorption of a compound to occur through a biological membrane the compound must be lipid soluble and the concentration on the inside of the membrane should be lower than on the outside. Compounds that are ionized at the pH of the biological environment will not normally be able to pass through the membrane by passive diffusion although they may be substrates for active transport processes.

(b) Plasma half-life ($t_{\frac{1}{2}}$) is the time taken for the concentration of a drug in the plasma (blood) to decrease by half from a given point. It reflects the rates at which the various *in vivo* dynamic processes of distribution, metabolism and excretion are taking place. It can be determined from a plot of the plasma level against time by measurement (see Figure 2.10) or from the equation:

$$\text{half-life} = \frac{0.693}{k_{el}}$$

where k_{el} is determined from the slope of the graph log plasma concentration vs time (slope $= -k_{el}/2.303$).

The half-life is an important measurement as changes in this parameter may reflect, for example, saturation of metabolism or excretion. A knowledge of the half-life is also important in relation to repeat dosing with a drug. If the dosing interval is shorter than the half-life then accumulation will occur. (See Figure 2.11.)

(c) Plasma clearance is a derived parameter and is an indication of the rate of removal of a drug from the blood or other body fluid by excretion or metabolism. It is calculated from the area under the plasma concentration vs time curve (AUC):

$$\text{clearance} = \frac{\text{dose}}{\text{AUC}}$$

Therefore, the units are volume/unit time, e.g. ml min^{-1}. Thus a plasma clearance of 100 ml min^{-1} means that 100 ml of plasma is completely cleared of the drug every minute. Therefore the higher the clearance, the more efficiently and rapidly a chemical is removed from the fluid.

(d) The term enterohepatic recirculation describes the process whereby a chemical in the body is secreted from the liver into the bile, passes into the small intestine and is then reabsorbed into the blood stream. For example, the chemical may be secreted into bile as a polar conjugate following metabolism in the liver. Then when the bile enters the intestine this conjugate is cleaved by bacterial metabolism and the original drug or other fragment is reabsorbed from the intestine and re-enters the liver via the portal circulation. (See Figure 2.13.) This process may be repeated several

times and therefore it prolongs the exposure of the liver and rest of the body to the compound. If the compound has been administered orally, very little may reach the systemic circulation. The plasma level profile may reflect the process by showing peaks at various times, corresponding to reabsorption, rather than a smooth decline.

Chapter 3

A1. (d) Altered chemical structure.

Metabolism by definition involves alteration of the chemical structure of a drug. Although increased excretion and decreased toxicity may often also occur this does not always happen and increased toxicity may result.

A2. (c) Is a central part of the drug metabolizing system.

Cytochrome P450 is the most important enzyme involved in drug metabolism. It is localized in the smooth endoplasmic reticulum and catalyses most of the phase 1 oxidation reactions.

A3. (d) The addition of an endogenous moiety.

Phase 2 metabolic transformations involve addition of a moiety derived endogenously which usually increases the polarity and water solubility. The moieties commonly involved are glucuronic acid, sulphate, glutathione and amino acids such as glycine.

A4. (b) A tripeptide.

Glutathione is composed of three amino acids: glutamic acid, cysteine and glycine (glutamyl-cysteinyl-glycine) abbreviated glu-cys-gly. It is involved in detoxication by conjugating with reactive metabolites, by reducing reactive metabolites and by reacting with and donating a hydrogen atom to free radicals.

A5. (a) True.

Cytochrome P450 catalyses phase 1 oxidation reactions.

A6. E.

All of these are involved in the operation of the microsomal enzyme system.

A7. (d) An inherited trait affecting a particular metabolic reaction.

The acetylation reaction in which the acetyl group (CH_3CO) is added to an amine, hydrazine or sulphonamide group is subject to genetic variation in humans. There are two phenotypes, rapid and slow acetylators which is a single gene trait governed by simple Mendelian inheritance with the rapid acetylator trait being dominant. This genetic trait results in a difference in the enzyme between the two phenotypes such that in the slow acetylators the enzyme, *N*-acetyltransferase (NAT2), catalyses the acetylation of substrates less efficiently than in the rapid acetylators. In the slow acetylators there are mutations in the gene coding for the enzyme, resulting in a relatively dysfunctional enzyme.

A8. (a) An increase in the synthesis of the enzyme.

Although the activity (and substrate specificity) of the enzyme may seem to be altered, in fact it is the synthesis of particular isozymes and their proportions which are altered. With some inducers liver weight and bile flow are increased, but not all inducers cause this.

SHORT ANSWER QUESTIONS

A9. (a) Dealkylation is the removal of an alkyl (usually a methyl or ethyl group) from a molecule. The alkyl group may be attached to a nitrogen, sulphur or oxygen atom as indicated below. The dealkylation reaction is catalyzed by the microsomal mono-oxygenase enzyme cytochrome P450 and involves an initial oxida-

tion of the alkyl carbon atom followed by a rearrangement with loss of the oxidized alkyl group as an aldehyde (e.g. methanal or ethanal as indicated below). The other product is either an alcohol, thiol or amine as shown below:

$$R-O-C_2H_5 \rightarrow R-OH + CH_3CHO$$
$$R-NH-CH_3 \rightarrow R-NH_2 + HCHO$$
$$R-S-CH_3 \rightarrow R-SH + HCHO$$

(b) Alcohol dehydrogenase is an enzyme found in many animal species that catalyzes the oxidation of alcohols to aldehydes. The coenzyme NADH is also required. There are several isoenzymes and a wide variety of alcohols are substrates. The enzyme is found particularly in the liver. There is evidence to suggest that there may be ethnic variations in the enzyme activity with Canadian Indians having reduced ability to metabolize ethanol.

(c) Glucuronic acid conjugation is the combination of certain foreign compounds with glucuronic acid to form glucuronides. Normally a carboxylic acid group or a hydroxyl group is conjugated to form ester or ether glucuronides. Occasionally thiol and NH glucuronides may be formed. The conjugates are water soluble and therefore readily excreted. The conjugation is catalyzed by one of a group of glucuronosyltransferases. Glucuronic acid is a six carbon carbohydrate molecule formed from glucose-1-phosphate. For conjugation it is combined with uridine diphosphate (UDP) as UDP-glucuronic acid. (See Figure 3.15.)

(d) Phase 1 metabolism refers to the first stage in the biotransformation of a foreign compound. The product will normally have a functional group added or an existing one modified that can be used as a 'handle' for a second endogenous group, derived from intermediary metabolism, such as glucuronic acid to be added in phase 2 metabolism. (See Figure 3.3.)

A10. (a) The ethnic background of a human individual may be an important determinant of their response to drugs and other chemicals. This may be due to a difference in sensitivity/susceptibility or to a difference in disposition. Glucose-6-phosphate dehydrogenase deficiency increases susceptibility to certain drugs such as primaquine, resulting in haemolytic anaemia. This deficiency is found in male individuals of a particular ethnic origin, such as those who inhabit or derive from the eastern Mediterranean such as Sephardic Jews from Kurdistan. Altered disposition in particular ethnic groups often occurs as a result of differences in enzymes. For example, the acetylator phenotype is differently distributed in Orientals compared with Egyptians, being mostly fast acetylators in the former but slow acetylators in the latter.

(b) The cytochrome P450 system, responsible for metabolizing many drugs, consists of many isozymes. These have different substrate specificities and there is variation in the activity of these isozymes between species and individuals. Therefore absence of a particular isozyme in an individual or species might make them more susceptible to drug toxicity if the particular isozyme was responsible for a detoxication pathway. Conversely, the individual or species might be less susceptible to a particular drug toxicity. The same also applies to susceptibility of a tissue to a particular drug toxicity, as isozymes vary in proportions between tissues within the same animal.

(c) The phenomenon of enzyme induction is the apparent increase in activity of an enzyme following the exposure of the animal to a xenobiotic. For example, repeated exposure to phenobarbital leads to an apparent increase in the activity of certain cytochrome P450 isozymes. This can be shown to occur with other enzymes involved with drug metabolism such as glucuronosyltransferase. The result is that the metabolism and therefore toxicity of a drug may be increased or decreased. This will depend on whether the drug or a metabolite is responsible for the toxic effect. For example, paracetamol toxicity to the liver is increased by induction of cytochrome P450 with phenobarbital in some species.

(d) Acetylator phenotype is a genetically determined characteristic in humans that determines the extent of acetylation of certain drugs. Isoniazid acetylation is affected by this phenotype. The phenotype is the result of a genetic difference between individuals resulting from mutations in the gene coding for *N*-acetyltransferase 2. Thus slow acetylators have less functional enzyme than fast acetylators. The result is that detoxication by acetylation of hydrazines, sulphonamides and amines is decreased in slow acetylators. Toxic effects such as hydralazine-induced lupus and isoniazid-induced peripheral neuropathy are more common in the slow phenotype.

Chapter 4

A1. (b) Dose.

Although all of the other factors may affect toxicity, the most important is the dose as toxicity is a relative phenomenon. Therefore at low enough doses there will be no toxic effect.

A2. (b) The liver metabolizes chemicals and this activity often makes it a target for toxicity. This may be due to the production of reactive metabolites or because its other metabolic activities are affected.

(c) The blood that is supplied to the liver is partly derived from the gut via the portal vein. Therefore chemicals absorbed from the gut will be presented to the liver. If toxic they may damage the liver tissue.

(d) The liver produces bile into which chemicals are excreted. Consequently, high concentrations of certain chemicals may occur in the bile and damage the bile duct. Alternatively, chemicals that are excreted into the bile via active transport, may, under conditions of high dosage, saturate the transport processes and accumulate in the liver, thereby causing damage.

A3. (e) Steatosis. Fatty liver or steatosis is the most common response of the liver to exposure to chemicals. This is because the liver is the primary site of fat metabolism in the mammalian body and this is easily disrupted by chemicals in various ways.

A4. (b) Immunosuppression. This is where the function of the immune system is reduced by a chemical such as dioxin or benzene which damages the thymus and bone marrow, respectively.

(d) Autoimmune reactions. These are immune-mediated reactions where the immune system attacks the structure of the body itself. An example is halothane-induced hepatic damage.

A5. (a) Death.

Although teratogens may cause all of these effects, especially growth retardation during the first stage of pregnancy before implantation, organogenesis or functional maturation, the fertilized egg is more likely to suffer death following exposure to a chemical.

A6.

Aneuploidization	loss or acquisition of a complete chromosome
Clastogenesis	loss, addition or rearrangement of parts of chromosomes
Mutagenesis	addition to or alteration of the number of base pairs

A7. (a) To indicate exposure has occurred. (d) To measure response and susceptibility. Biomarkers are biochemical indicators of the exposure, the response or the susceptibility to chemicals.

SHORT ANSWER QUESTIONS

A8. Direct toxic action: tissue lesions e.g. paracetamol-induced liver damage; biochemical lesions, e.g. fluoroacetate interference with the TCA cycle leading to death from cardiac arrest; pharmacological/physiological effects, e.g. malathion causing exaggerated effects of acetylcholine; immunotoxicity, e.g. allergic responses caused by exposure to penicillin; teratogenicity, e.g. thalidomide-induced birth defects; genetic toxicity, e.g. 5-bromouracil becomes incorporated into replicating DNA and this leads to mutations (base-pair transformations); carcinogenicity, e.g. vinyl chloride and aflatoxin both cause liver cancer.

A9. Immunosuppression, autoimmune responses, hypersensitivity and allergic reactions. Immunosuppression is where the function of the immune system is reduced by a chemical such as dioxin or benzene which damage the thymus and bone marrow, respectively. Autoimmunity is where the immune system damages or destroys the structure of the body itself such as occurs in halothane hepatoxicity. Hypersensitivity is where the immune system recognizes that a chemical is foreign and mounts a response involving antibodies in a humoral response or lymphocytes in a cell-mediated response. Hydralazine-induced lupus is a response in which antibodies and an antibody–antigen complex is formed. An allergic reaction is where the immune system recognizes that a substance is foreign and an immune reaction is mounted, for example as occurs with foreign proteins such as pollen or those devised to be used as drugs.

A10. The first stage is initiation, in which the chemical or a reactive metabolite interacts with DNA. The second stage is promotion in which the initiated cell undergoes division and a clone of initiated/altered cells is produced. Finally, the third stage is where the clone of cells undergoes progressions in which the neoplastic cells may become a malignant tumour, growth increases and the cells become malignant and invade tissue.

Chapter 5

A1. E.

After overdoses or inappropriate doses of a drug the pharmacological effect may be exaggerated, for example excessive lowering of blood pressure. This is an adverse effect. Sometimes a drug may cause an adverse effect after therapeutic doses in particularly susceptible individuals. This is an idiosyncrasy. Inappropriate doses of a drug may cause unwanted effects not related to the expected/desired pharmacological effect of that drug. Occasionally there may be an interaction between a drug and a dietary constituent such as between monoamine oxidase inhibitor drugs and amines in foodstuffs such as cheese.

A2. (d) Metabolic activation by the microsomal enzymes.

Although (a) and (c) are also true, metabolic activation to a reactive benzoquinoneimine, which interacts with tissue components, is the cause of the toxicity. (See Figure 5.1.)

A3. (a), (b), (c). There are a number of predisposing factors in hydralazine toxicity. These include the acetylator phenotype, gender and dose. The toxicity or adverse effect is almost exclusively confined to the slow acetylator. The toxicity is more common in females than males. The adverse effect is more likely to occur at higher doses (although the severity of the effect does not seem to be related to the dose).

A4. (c) Thalidomide was a sedative drug used for the relief of morning sickness in pregnant

women. It caused malformations in babies born to mothers who took the drug during the susceptible period (3rd to 8th week of pregnancy). The malformations were shortened arms and legs, known as phocomelia. The S isomer of the drug is more active than the R isomer. Pregnant rats were not susceptible to this effect.

A5. (c) Halothane, an anaesthetic drug may occasionally cause severe liver damage in patients. This is more common in females than males. The mechanism involves metabolism by cytochrome P450 and production of an antigenic conjugate. The immune response involves T-lymphocytes as well as antibodies. It is an autoimmune reaction. (See Figure 5.5.)

SHORT ANSWER QUESTION

A6. Aspirin is mainly metabolized to salicylate. The distribution of salicylate into tissues and its excretion into urine are sensitive to pH. This is because only the ionized form of salicylate enters tissues and especially the brain. However, the brain in particular is sensitive to the toxic effects of salicylate. When plasma pH drops, as a result of salicylate poisoning, this distribution into the brain increases. Similarly, excretion into urine is reduced if urinary pH becomes more acidic. Raising the pH of plasma and urine with bicarbonate is therefore the central part of treatment of aspirin poisoning. (See Figure 5.3.)

Chapter 6

A1. (c) Dermatitis is a common reaction to the metal nickel.

A2. (a), (b), (c) and (d) are all known carcinogens. Cadmium is not known to be a carcinogen in man but asbestos, 2-naphthylamine and vinyl chloride are associated with lung cancer (mesothelioma), bladder cancer and liver cancer (haemangiosarcoma) in man, respectively.

A3. (c) The NOAEL or No Observed Adverse Effect Level is needed to determine the Threshold Limit Value (TLV) for an industrial chemical. This is unusually divided by a safety factor to give the TLV. The latency period for the effect and half-life would be included in the NOAEL. The daily exposure level may be the same as, less or more than the TLV depending on the particular circumstances.

A4. The toxicity of asbestos is affected by (a), (b), (c), (d) and (e).

A5. (c) Both vinyl chloride and cadmium affect bones, albeit in different ways.

SHORT ANSWER QUESTION

A6. 2-Naphthylamine undergoes hydroxylation on the nitrogen atom catalyzed by cytochrome P450 and the N-hydroxy product is conjugated with glucuronic acid. This conjugate is excreted into the urine but is relatively unstable and under the acidic conditions of the urine is hydrolyzed to yield a reactive metabolite. This reacts with DNA in the cells of the bladder that are exposed leading to the formation of cancer. However, 2-naphthylamine is also acetylated on the nitrogen atom and this is a detoxication reaction as hydroylation and reactive metabolite formation do not subsequently occur. Therefore the fast acetylator phenotype individual is less at risk than the slow acetylator.

Chapter 7

A1.

Erythrosine	colouring agent
Monosodium glutamate	flavour enhancer
Cinnamaldehyde	flavour
Butylated hydroxytoluene	anti-oxidant
Benzoyl peroxide	bleach

A2. Tartrazine is a colouring agent which may cause urticaria (b) and is reduced by gut bacteria (c). It is also known as E102. Tartrazine sensitivity is often related to aspirin tolerance.

A3. (a), (d) and (e) are true. Saccharin may cause bladder tumours in rats at very high doses. Saturation kinetics occurs at high doses, which may account for the bladder tumours at these doses. It has low toxicity and although it was banned from use under the Delaney Clause for a while it is now allowed for use.

A4. (d) Is true, (a),(b) and (c) are false. Adulterated rape-seed oil was sold for use as cooking oil. The rape-seed oil was for industrial use and had been adulterated with the addition of aniline. There were a number of symptoms including pulmonary oedema and muscular atrophy.

SHORT ANSWER QUESTION

A5. Aflatoxin. These are toxins produced by a mould (*Aspergillus flavus*) that grows on foodstuffs such as peanuts when stored in damp, warm conditions. Aflatoxin B_1 is a potent liver carcinogen. The mechanism involves metabolism to a chemically reactive intermediate, an epoxide, which interacts with DNA.

Ptaquiloside. This is a toxin which occurs in naturally in edible bracken fern shoots. It causes throat cancer in humans. In animals it causes intestinal and bladder cancer. A breakdown product of ptaquiloside is responsible, reacting with adenine in DNA and causing DNA strand breakage.

Botulinum toxin. This is one of the most potent toxins known. It is produced by the anaerobic bacterium *Clostridium botulinum*. It may contaminate tinned and bottled food, although it is destroyed by heating. It acts by binding irreversibly to the nerve terminals, preventing the release of acetylcholine. This causes paralysis and fatal cessation of breathing.

Chapter 8

A1. (b) and (d) are true; (a), (c), (e), (f) and (g) are false.

DDT is an organochlorine pesticide, which has low mammalian toxicity and does not destroy plants. Unlike organophosphates it does not inhibit cholinesterases. It is not directly toxic to eggs although it may contribute to their breakage as a result of eggshell thinning.

A2. Parathion is an organophosphate insecticide which is toxic to humans. Parathion is metabolized to paraoxon which inhibits cholinesterases leading to elevated levels of acetylcholine which causes excessive cholinergic stimulation and symptoms such as bronchoconstriction. (See Figure 8.4.)

True: (d) and (e). False: (a)–(c).

A3. Paraquat

True: (c) and (d). False: (a), (b), (e) and (f).

Paraquat is a bipyridyl herbicide. It is actively taken up into lung tissue by the putrescine uptake system. It is concentrated in the lungs where it causes lipid peroxidation, the production of active oxygen species and fibrosis. The active oxygen species are detoxified by superoxide dismutase (SOD) but this is over-

whelmed when large amounts of paraquat are ingested. (See Figure 8.6.)

SHORT ANSWER QUESTION

A4. Fluoroacetate is toxic because it is incorporated into intermediary metabolism, being first converted to fluoroacetyl CoA. This is incorporated into the tricarboxylic acid cycle, forming fluorocitrate. This analogue of citrate, however, cannot be further metabolized by the next step to cis-aconitate as the fluorine atom cannot be removed. Therefore the tricarboxylic acid cycle is blocked, ATP production compromised and mammals die of heart failure.

Chapter 9

A.1 True: (b), (c), (e). False: (a), (d).

Inorganic lead is present in cigarette smoke and can be absorbed as particles of lead oxide for example through the lungs. It interferes with the synthesis of haem, some of which occurs in the mitochondria. The result is a reduction of haemoglobin which leads to damage to red cells. Organic lead such as that added to petrol, is toxic to the central nervous system.

A2. (a), (b), (c) are not true; (d) and (e) are true.

Mercury binds to SH groups and organic mercury has a long half-life. Metallic mercury easily vaporizes and the vapour can be readily absorbed where it is toxic to a variety of tissues including the central nervous system. Inorganic mercury is especially toxic to the kidney.

A3. (f) is true; (a), (b), (c), (d) and (e) are false.

The great smog in London was in 1952. Photochemical smog contains ozone, nitrogen oxides and hydrocarbons. Ozone is an irritant, toxic gas. PM10 is the name of very small particles (diameter less than 10 μm) that are believed to be responsible for lung disease. Acid rain results from the production of sulphur dioxide and nitrogen oxides. It is caused by the wet precipitation of sulphuric and nitric acids and the dry precipitation of sulphur dioxide and nitrogen oxides.

Reducing smog is largely the result of burning of fossil fuels and has high levels of sulphur dioxide and particulates.

A4. True: (c) and (e). False: (a), (b) and (d).

DDT is an insecticide, not especially toxic to birds or chicks directly. However, its metabolite DDE is responsible for causing eggshell thinning. This results in egg breakage and loss of chicks. By killing insects of many types, DDT also reduces the food supply of some birds.

SHORT ANSWER QUESTION

A5. Bioaccumulation is the accumulation of a chemical substance in a biological organism. This is usually a reflection of the lipophilicity of the compound.

Biomagnification is the process whereby the concentration of a chemical substance in the organisms in a food chain increases towards the top of the chain. Thus, the predator at the top of the food chain will have the highest concentration of pollutant.

For a compound to bioaccumulate it should be lipid soluble rather than water soluble. For example, the pesticide DDT will bioaccumulate in organisms exposed to it as it dissolves in the fat in adipose tissue. Second, the compound should be resistant to metabolism and therefore poorly excreted so that it is eliminated slowly from the organism. For example, polybrominated biphenyl compounds, such as those that contaminated livestock and people in Michigan in 1973, are very resistant to metabolism, are eliminated extremely slowly and so

have very long half-lives. Continued exposure to such compounds will therefore result in accumulation in fat tissue.

Chapter 10

A1. True: (c) and (e). False: (a), (b) and (d).

Pyrrolizidine alkaloids are found in plants such as *Heliotropium* species. These compounds cause liver and lung damage. *Amanita phalloides* is a toxin found in the Death Cap mushroom. It causes liver damage which may be fatal. Botulinum toxin is produced by the anaerobic bacterium *Clostridium botulinum* and is highly toxic, causing paralysis. Ricin is the most toxic substance known and is found naturally in the castor bean. Fluoroacetate occurs naturally in certain plants in South Africa and Australia.

A2. True: (b) and (d). False (a) and (c).

Tetrodotoxin is a toxin found in the Puffer fish and Californian newt. It is highly potent, causing muscle paralysis. The toxin selectively blocks sodium channels along the axon, blocking transmission of the action potential.

SHORT ANSWER QUESTION

A3. Botulinum toxin causes irreversible blockade of the motor nerve terminal at the myoneural junction. This prevents the release of acetylcholine and the muscle behaves as if denervated. The victim therefore suffers paralysis and may have difficulty in breathing which can be fatal if severe.

Chapter 11

A1. True: (b). False: (a), (c), (d) and (e).

Ethylene glycol toxicity requires metabolism by the enzyme alcohol dehydrogenase. Ethanol is also metabolized by this enzyme and so when administered to a poisoned patient ethanol competes for the enzyme and blocks the metabolism of the ethylene glycol.

A2. True: (b), (d). False: (a), (c), and (e).

Carbon monoxide binds to haemoglobin more strongly than oxygen and forms carboxyhaemoglobin. The lack of oxygen in the tissues results in damage, especially to those that have a high demand for oxygen such as the brain and heart. Death is usually due to respiratory failure. Carbon monoxide will also bind to other haem proteins such as cytochromes. The lack of oxygen leads to anaerobic respiration and hence lactic acidosis.

A3. True: (a), (b). False: (c), (d) and (e).

Ethylene glycol is a dihydric alcohol which is metabolized by alcohol dehydrogenase to an aldehyde and then (via aldehyde dehydrogenase) subsequently to an acid. The final product is oxalic acid. This may crystallize in the brain. The increased production of NADH as a result of metabolism leads to excessive production of lactic acid and the presence of acidic metabolites causes acidosis.

A4. True: (a) and (f). False: (b), (c), (d), (e).

Some solvents may sensitize the myocardium leading to sudden death from heart attack in apparently healthy young people who have engaged in solvent abuse.
Alcohol depresses the central nervous system, especially at high doses. Alcohol is metabolized to acetic acid. The major target for methanol is the eye – it causes blindness. Large doses of alcohol lower blood sugar (hypoglycaemia).

A5. (b), (d) and (e). Dicobalt edetate, a chelating agent, is used as an antidote to cyanide poisoning. N-acetylcysteine is used as an antidote for paracetamol overdose as it helps to

restore the glutathione that is depleted by paracetamol. Pralidoxime is used as an antidote to organophosphate poisoning, as it binds the organophosphate in preference to the acetylcholinesterase enzyme.

SHORT ANSWER QUESTION

A6. General treatments for poisoning include the use of emetics to cause vomiting and so rid the stomach of the poison; gastric lavage to wash the poison out of the stomach; absorbants which are given orally to the patient and absorb the poison in the stomach. Enhancing excretion may be used which involves administration of aqueous solutions by mouth or intravenously (forced diuresis) to increase urine flow. If bicarbonate or ammonium chloride are included in the aqueous fluid then the pH of the urine is made more basic or acidic, respectively. This change will facilitate the excretion of acids or bases, respectively.

Extraction of the poison from the blood may be used in severe cases and involves either haemoperfusion or haemodialysis.

Chapter 12

A1. True: (c) and (d). False: (a) and (b).

Acute toxicity studies are to define the toxicity and determine a dose–response relationship. Sub-chronic studies are for the determination of short-term repeated exposure. Ecotoxicity studies use the small water organism *Daphnia* as a test species. Teratogenicity studies are for the determination of the effect of a compound on the developing organism *in utero*.

A2. True: (a), (b), (c) and (d). False: (e), (f) and (g)

SHORT ANSWER QUESTIONS

A3. The following are the seven questions:

1 Is it a novel compound or has it been used before?
2 Is it to be released into the environment?
3 Is it to be added to human food?
4 Is it to be given as a single dose or repeatedly?
5 At what dosage level is it to be administered?
6 What age group will be exposed?
7 Are women of childbearing age likely to be exposed?

A4. The four types of epidemiological studies are: cohort studies, case-control studies, cross-sectional studies, ecological studies.

A5. The three Rs relate to the use of animals in toxicity testing. They are *replacement* of animals with alternatives such as *in vitro* systems; *reduction* of numbers of animals used by careful design of experiments and *refinement* of the techniques used to ensure greater animal welfare.

A6. ***Risk*** is defined as the probability that a hazard will cause an adverse effect under specific exposure conditions.

Risk may also be defined in the following way:

Risk = hazard × exposure.

Hazard is defined as the capability of a substance to cause an adverse effect. Conversely safety may be defined as 'the practical certainty that adverse effects will not occur when the substance is used in the manner and quantity proposed for its use'.