BIOCHEMISTRY of XENOBIOTICS
Towards a Healty Lifestyle
and Safe Environment

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Contents

ABSTRACT

 𝒳 enobiotics or foreign compounds include products of industry such as pesticides, industrial chemicals, synthetic drugs, antibiotics and heavy metals. Natural xenobiotics include animal poisons and toxins, antibiotics, drugs and toxic products from plants. These compounds may cause damage to living organisms resulting in deformities, DNA damage, poisoning or at the very least, a feeling of discomfort. In an environment contaminated by all these compounds, living organisms have managed to adapt by changing the way it reacts to the effects of these foreign compounds. They may modify the function of existing enzymes to catalyse reactions of xenobiotics or physically move to a less contaminated location. The cell as the smallest unit of life contains a diverse array of systems that effectively react to xenobiotics. This paper focuses on the biochemistry of the cell reacting to selected xenobiotics citing work done on insects, rats and mice and bacteria, in an effort to understand the underlying principles of xenobiotic metabolism.

INTRODUCTION

A foreign compound or xenobiotic is defined as a chemical compound or substance found in an organism but which is not normally produced or expected to be present in that particular organism (Williams, 1959). A foreign compound that readily comes to mind is penicillin. Although it is an antibiotic produced by a bacterium, it is not normally present in other bacteria or other living organisms.
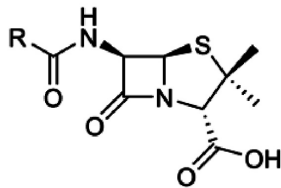
Core structure of penicillin

   Other xenobiotics may be drugs, toxic substances and poisons, whether naturally occurring or synthetic, heavy metals, toxins or hazardous chemicals such as those used in agriculture namely insecticides, pesticides, herbicides and inorganic fertilizers. Drugs and antibiotics are classified as xenobiotics to humans because the human body does not produce these chemicals. Further, these chemicals are not present in the normal human diet. Naturally produced compounds can become xenobiotics to other animals for example the intake of human hormone residues by fish living downstream of sewage treatment plant outfalls. Predatory animals can also absorb xenobiotics such as venoms and other defense chemicals produced by its prey. Humans may also absorb xenobiotics from consuming food adulterated with substances such as artificial growth hormones.
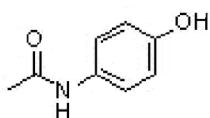
	Acetaminophen (Paracetamol). Acta as an analgesic. Known to deplete glutathione levels in liver.
	Ampicillin. A broad spectrum antibiotic.
	Cocaine. A hallucinogenic drug.
	Nicotine. Major chemical compound of cigarette smoke. Attributed to cigarette addiction. Possesses insecticidal activity.
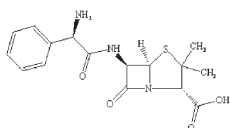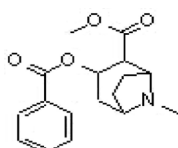
Figure 1 Examples of drugs and antibiotics in common use

Xenobiotics pose a problem to the environment. Many of them, such as industrial chemicals which include DDT and chlorinated biphenyls, are persistent and difficult to degrade. They may remain present in the environment for a long time and thus become distributed to all components of the ecosystem, including the food chain. Theses chemicals may be endocrine disruptors, meaning they can cause havoc to the hormone systems of organisms by imitating the action of hormones. The appearance of deformed frogs, a reduction of reproductive

capability in amphibians and brittle eggs are clear examples of xenobiotic contamination in the environment (Figure 2). Alternatively, xenobiotics may directly cause deformities in the egg, resulting in the birth of deformed babies as exemplified by the effect of the anti-morning sickness drug, thalidomide.
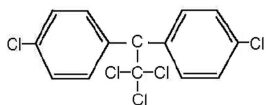
	DDT. [Dichloro-Diphenyl-Tricchloroethane]. An organochlorine insecticide. Causes thinning of egg-shells. An environmental estrogen. Half-life of 5 years. LD_{50} (rat) of 113 mg/kg body weight . Banned in the 1980s.
	Parathion. An organophosphate insecticide. Half-life of 1-12 weeks. LD_{50} (rat) of 4 mg/kg body weight. Acutely toxic to mammals.
	Temik. A carbamate insecticide. Half-life of 1- 8 weeks. LD_{50} (rat) of 1 mg/kg body weight. Acutely toxic to mammals. Leaches into groundwater.
	2,4-D. (2,4-Dichlorophenoxyacetic acid) A weedicide. Kills all plants. Mixed with 2,4,5-T to form agent orange. Can have dioxins as a contaminant. Half-life of 3 months. LD_{50} (rat) of 370 mg/kg body weight.
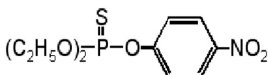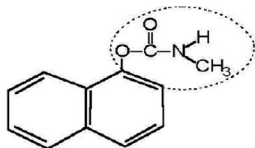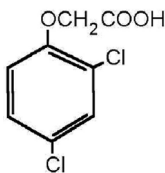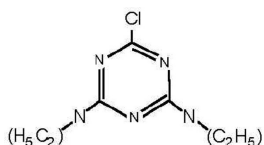
Atrazine.

Most widely used herbicide in the world. Mixed with 2,4,5-T to form agent orange. Can have dioxins as a contaminant. Half-life of 1-2 years. Suspected carcinogen. Acutely toxic to fish. LD_{50} (rat) of 3000 mg/kg body weight.

Figure 2 Some examples of pesticides in use

The living organism is constantly in contact with xenobiotics either through exposure to these compounds in the environment, or through nutrition. Thus the living organism, either through adaptation or already naturally endowed, possesses various means to deal with xenobiotics. This is clearly seen in the varying toxicities of certain pesticides on their target organisms. For example, various insect species demonstrate different LC_{50} (the lethal concentration of an insecticide required to kill 50% of a given insect population) values for a given insecticide. If an insecticides's LC_{50} value is low for a particular insect species, it means that it is more toxic or effective in killing that particular insect species than another insecticide with a higher LC_{50} value. Thus, values for lethal dose of insecticides need to be established for target and non-target organisms.

Long term use of drugs may also lead to adverse effects. Danazol for example, is a synthetic hormone used in the treatment of endometriosis while Clomiphene, a non-steroidal antiestrogen, is used to stimulate ovulation in women suffering from amenorrhea. Prolonged treatment using both danazol and clomiphene has been reported to cause cancer of the liver.

SAFE AND EFFECTIVE PESTICIDES

An effective insecticide, as an example of pesticides, will be one that kills insects at very low doses and at the same time cause no adverse effects at very high

doses to non-target organisms. An ideal situation would be for an insecticide to exhibit selective killing; where only one particular insect species is targeted while other species are relatively unaffected. However, with most insecticides, this is not the case. Due to the mode of killing, almost all species are affected. For example, the insecticide endosulfan, while targeting insects, is also very toxic to fish. Hence, in paddy fields, freshwater fish which form a subsistence supply of proteins to farmers, are depleted after application of the insecticide (Shamaan *et al,* 2005).

Endosulfan is toxic to both insect and fish due to its effectiveness in inhibiting nerve impulse transmission. It inhibits the enzyme acetylcholinesterase, which breaks down the neurotransmitter acetylcholine to choline and acetic acid. In animals, acetylcholine is translocated across the synaptic junction when a nervous impulse is transmitted. It is rapidly broken down by the enzyme acetylcholinesterase so that the impulse can be stopped. The breakdown products, acetic acid and choline will then be recycled and reacted upon to form acetylcholine to be used in future transmission. Stopping this cycle by inhibiting the enzyme is a good way of stopping nerve transmission. Paralysis and eventually, death will occur.

Another consideration for a safe and effective insecticide would be that it does not remain persistent in the environment and will cause no ill-effects to human beings. However, some caution is necessary in detailing considerations of an effective insecticide. There is no such thing as an ideal synthetic insecticide (or pesticide) if we are to refer to The 5th Edition of Truman's Scientific Guide to Pest Control Operations (1997) which describes the ideal pesticide as :

> *"Ideally any pesticide will act rapidly on pests, yet be completely harmless to people, domestic animals, wildlife, and other aspects of the environment. Its residues would only last as long as was necessary to create the desired effect, usually for very short periods. It would also be inexpensive and readily available*

in necessary quantities, chemically stable (before application), non-flammable, and otherwise safe to use around homes or industrial sites. It would be easily prepared and applied, non-corrosive and non-staining, and it would have no undesirable odor."

Scientifically, pesticides are not considered safe since they is meant to kill pests. Additionally, most pesticides demonstrate adverse effects at non-lethal concentrations such as, uncontrolled movement in fish exposed to very low doses of insecticides, or a change in the gross morphology such as skin discolouration in catfish after exposure to sub-lethal doses of the insecticides carbofuran and endosulfan (Figure 3). Skin discolouration was more pronounced in the endosulfan-treated catfish than on those treated with carbofuran, as compared with the normal control catfish. Growth and development of fish living in areas subjected to sub-lethal concentrations of carbofuran and endosulfan were also retarded (Abu Zeid *et al,* 2004).

INTERACTION OF XENOBIOTICS WITH LIVING ORGANISMS

Xenobiotics may interact with cellular components in the cell. They may cause apoptosis (programmed cell death; a damaged cell undergoes a programme leading to its death) at very low levels or necrosis (immediate cell death) at toxic levels. Important components of the cell that can interact with xenobiotics include proteins, lipids and DNA, the latter leading to mutation and the possibility of cancer formation. The insecticide DDT has been found to accumulate in fatty tissues of animals including humans, and egg shells of predatory birds (Rogan and Chen, 2005). It displayed endocrine disrupting activity, meaning it imitated estrogens in animals, in addition to causing preterm birth and early weaning, and possible disruption in semen quality, menstruation, gestational length and duration of lactation in humans.
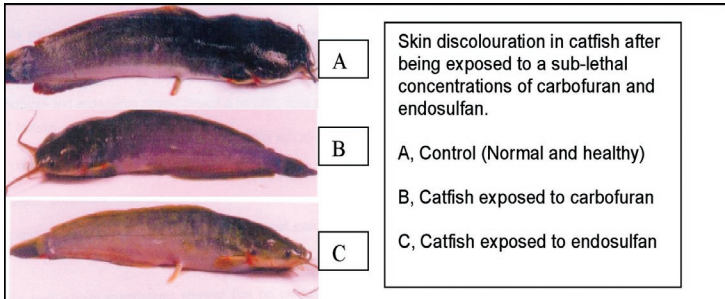
Figure 3 Skin discolouration in catfish exposed to carbofuran and endosulfan. The catfish (ikan keli) showed skin discolouration after being treated with sub-lethal doses of carbofuran and endosulfan. Skin discolouration was more pronounced with endosulfan exposure (C) than carbofuran (B), and as compared with the normal control catfish (A)

Living in a contaminated environment, the living organism will need systems that are capable of coping with the effects of xenobiotics on biological processes; either to isolate the body from the environment or when that is not possible, to react on the xenobiotic and render it harmless or excrete it. Briefly, there are two ways in which the body deals with xenobiotics: the immune system and enzymes.

THE IMMUNE SYSTEM

Animals (and humans) possess several ways of dealing with xenobiotics. The blood contains proteins known as antibodies which are commonly called immunoglobulins. The antibodies interact with specific xenobiotics to prevent them from exerting harmful effects on the body. Normally, the immunoglobulins react with a class of substances called antigens, substances that may activate the cells to produce antibodies. Bacteria, viruses, venoms, toxins and other substances produced by other living organisms which can cause harm to the

body can be classified as antigens. Several other chemical compounds such as aflatoxins, a toxin produced by the fungus *Aspergillus flavus*, and pesticides, are not antigens because they do not cause the cells to produce antibodies.

There are many types of immunoglobulins and all of them are specific in their actions. For example, an antibody to cobra snake venom may not react against the venom of the viper. Therefore, many antibodies may need to be produced by the body against a whole range of antigens. Upon exposure to the various antigens, the body keeps a record of these exposures and stores the memory in special cells known as the B- and T-cells of the lymphatic system. These cells may readily produce the antibodies in sufficient amounts to react against the invasion of a particular antigen.

Defence System at the Molecular Level: Enzymes

In addition to the immune system, the body possesses yet another system to deal with xenobiotics. This system is carried out at the molecular level in the cell. Since most xenobiotics are small molecules that do not activate the immune system, the body cannot utilize the immune system to counteract these foreign compounds. Thus, when the body comes in contact with these molecules, the body needs to engage other systems to deal with the harmful characteristics of these molecules.

In the cell, there are a number of enzyme systems that can be specifically employed to deal with harmful molecules. These are known as detoxication enzymes. Essentially, these enzymes function to catalyse reactions that lead to the deactivation of xenobiotics harmful to the cell, rendering them harmless. Subsequently, the xenobiotics will be prevented from participating in any further metabolic reactions or be removed from the cell by excretion.

The enzyme systems involved in detoxication are enzymes involved in normal intermediary metabolism i.e. biological processes occurring in the cell to produce energy and new building blocks for normal cell functions such as

growth and production of new products. In the presence of xenobiotics, these enzymes take on the additional role of reacting on the xenobiotics to produce products that are not harmful to the cell, possibly to be utilized in the same way as normal products.

DETOXICATION MECHANISMS

There are three major steps in the detoxication mechanism. The first step involves reactions that transform harmful molecules from a lipophilic to more hydrophilic in nature. This is called a biotransformation reaction, also known as the Phase I reaction. The second step is known as the conjugation reaction or Phase II reaction and the third step involves reactions leading to the ultimate excretion of the xenobiotic from the cell, also known as the Phase III reaction.

Biotransformation – Phase I Reaction

Most xenobiotics are lipophilic in nature, meaning that they are soluble in oil or lipids, and because of this, they may readily cross the cell membrane and cause damage inside the cell. At the same time they are difficult to excrete from the cell or system because they are insoluble in water. Therefore, they may persist in the system and cause damage to the cells for a longer period of time.

There are three major types of reactions occurring in Phase I or biotransformation. These are:

i) oxidation reactions catalysed by a number of enzymes such as the cytochrome P_{450} monooxygenase system, Flavin-containing monooxygenase system, alcohol dehydrogenase, aldehyde dehydrogenase, monoamine oxidase and lastly, the peroxidases

ii) reduction reactions catalysed by NADPH cytochrome P450 reductase and reduced cytochrome P_{450}

iii) hydrolysis catalysed by esterases, amidases and hydrolases

A series of biochemical reactions may occur to the xenobiotic once it enters the cell. It may undergo oxidation reaction in which an oxygen atom is added to the molecule resulting in an electron-loving molecule (electrophile) such as a peroxide or an oxide. The xenobiotic may also undergo hydrolysis or reduction reactions which would result in the formation of a nucleophile (electron-rich molecule) such as a hydroxylated xenobiotic or a sulphydryl or an amine (Figure 4).
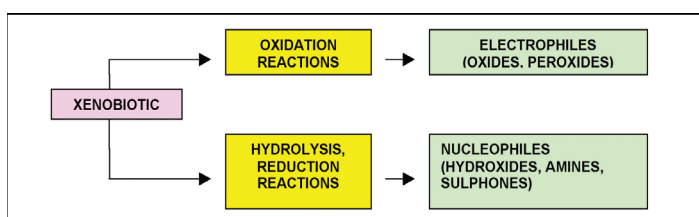
Figure 4 The reactions and products in the biotransformation of a xenobiotic in a cell.

The end-products of biotransformation reactions are charged molecules - they are either electrophilc or nucleophilic as compared with before the biotransformation reactions. One of the most common biotransformation reaction is the addition of polar groups to the foreign molecule i.e. addition of a hydroxyl group (-OH) catalysed by the cytochrome P_{450} dependent mixed function oxidase system. This multi-enzyme system acts to add an atom of oxygen into a non-activated xenobiotic, notably a hydrocarbon molecule, to produce a more polar molecule (Guengerich, 2001). The product of this reaction is the hydroxylated xenobiotic, which is highly reactive and more polar than the starting xenobiotic. The reaction mechanism of the cytochrome P_{450} mixed function oxidases proceeds through the reduction of cytochrome-bound oxygen and the generation of a highly-reactive species (Schlichting *et al,* 2000), as outlined in the reaction below;

$$R + NADPH + H^+ + \tfrac{1}{2}O_2 \rightarrow ROH + NADP^+ + H_2O$$
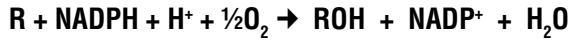
where R, a lipophilic compound, after biotransformation becomes a reactive hydroxylated compound, ROH.

Besides the addition of an oxygen atom to the foreign molecule, the cell is capable of adding a sulphur (-S) and nitrogen (-N) atom in this reaction. This will result in the dealkylation (removal of alkyl groups such as methyl or ethyl group from the xenobiotic) by addition of a sulphydryl group (-SH) or an amino group (-NH). The products of biotransformation reactions are normally electrophilic or nucleophilic in nature, such as sulphides and amines. The products of biotransformation reactions such as the oxides, hydroxylated metabolites, sulphides and amines are now primed for conjugation reactions.

Conjugation – Phase II Reactions

The second step in the detoxication mechanism involves the conjugation of the biotransformed xenobiotics to cellular endogenous compounds abundantly available in the cell. The second step is also known as the Phase II reactions. The cellular compounds involved in these conjugation reactions are mainly glutathione, amino acids and glucuronic acid. The enzymes which catalyse the reactions of these compounds with xenobiotics are also those involved in normal cellular metabolism. These conjugation reactions lead to rendering of the xenobiotics from being lipophilic to becoming more strongly hydrophilic in nature, and resulting in them being more readily and easily excreted from the cell (Figure 5).
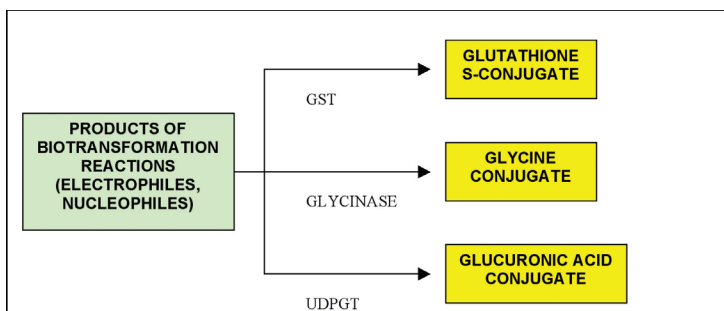
Figure 5 Conjugation reactions. The enzymes involved in the conjugation reactions are glutathione S-transferase (GST), glycinase and UDP-glucuronyltransferase (UDPGT)

Glutathione (GSH) is a tripeptide i.e. a molecule comprising three amino acids, glutamic acid, cysteine and glycine, linked by covalent bonds. Glucuronic acid is an acidic derivative of glucose. Being readily available and abundant in the cell, glutathione, glycine and glucuronic acid are all anionic and obvious choices for conjugation reactions. The conjugation reactions are catalysed by a large group of broad-specificity transferases, which in combination can metabolise almost any hydrophobic compound that contains nucleophilic or electrophilic groups (Jakoby and Zeigler, 1990).

One of the most important conjugation enzymes is the glutathione S-transferases (GSTs). This group of enzymes catalyses the conjugation of glutathione which acts as a large anionic group, to a wide variety of hydrophobic and reactive electrophilic compounds (Figure 6). As a result of the conjugation reaction, the hydrophobic xenobiotic molecule will contain a large anionic group (glutathione), making it a polar compound. The conjugate is then not able to diffuse freely across the cell membrane and may require energy for transfer out of the cell (Konig *et al,* 1999).
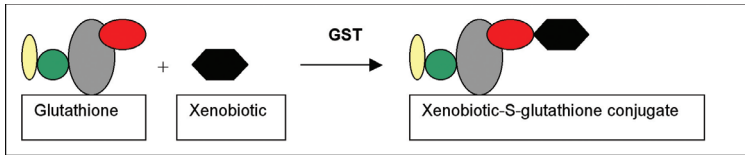
Figure 6 Conjugation of a xenobiotic with glutathione catalysed by the enzyme glutathione S-transferase. Glutathione forms a sulphide bond through its sulphydryl group (red sphere) with the xenobiotic (black hexagon)

Mercapturic acid pathway – Phase III Reactions

The third step in the detoxication mechanism involves reactions leading to the removal or excretion of the conjugated xenobiotics from the cell. The process (Phase III reactions) is briefly described in Figure 7 using the mercapturic acid pathway for the excretion of the xenobiotic from the cell. Three enzymes are involved in the process; firstly, gamma-glutamyltranspeptidase (GGT) which catalyses the cleavage of glutamic acid residue of glutathione, secondly, a dipeptidase which cleaves the glycine residue and thirdly, N-acetyltransferase which attaches an acetic acid group to the remaining xenobiotic conjugate. The acidified conjugate is finally excreted from the cell as a mercapturic acid. After the conjugation reactions, the xenobiotic metabolites may be subjected to mercapturic acid synthesis. In this pathway, the gamma-glutamyl and glycyl residues of glutathione are removed by GGT and dipeptidase respectively, to yield the cysteinyl metabolite of the xenobiotic. The cysteinyl metabolite is then acetylated by N-acetyltransferase and the xenobiotic residue is finally excreted from the cell (Commandeur *et al,* 1995). Most of the glutathione conjugates go through this path to yield N-acetylcysteine or mercapturic acids (Boyland and Chasseaud, 1969).
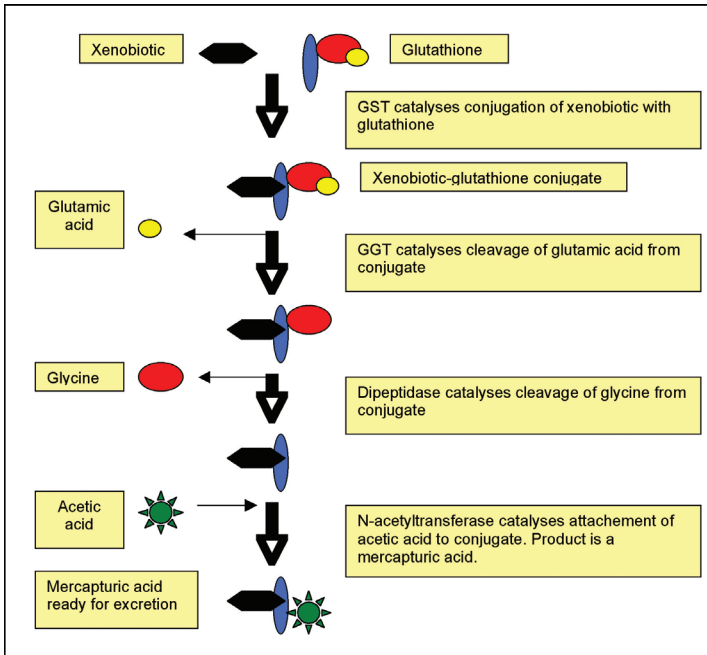
Figure 7 Mercapturic acid formation. The process begins with the cleavage of the glutamic acid residue (yellow sphere) by gamma-glutamyl transpeptidase, followed by cleavage of glycine residue (red sphere) by a dipeptidase leaving the xenobiotic (black hexagon) conjugated with cysteine (blue sphere). An acetate group (green star) is attached to the cystine residue by N-acetyl transferase before the conjugate is excreted from the cell.

THE ROLE OF CONJUGATION ENZYMES IN THE METABOLISM OF XENOBIOTICS

Amongst the many conjugation enzymes functioning in the cell, it is the glutathione S-transferases (GST) which have been intensively researched and documented. The action of the enzyme in mercapturic acid synthesis was first reported by Habig *et al,* (1974) and since then, detailed mechanisms of the enzyme, including its origins, have been elucidated.

The enzyme plays an important role in the metabolism of insecticides. Resistance to a particular insecticide such as the organophosphates has been attributed to the action of the GSTs where resistant houseflies usually have higher enzyme activities than the more susceptible strains (Motoyama *et al,* 1980). Taking the well known insecticide DDT [1,1,1-trichloro-2,3-bis(p-chlorophenyl)ethane] as an example, it has been shown that the housefly can rapidly metabolise the insecticide rendering it non-effective to the housefly. It was shown that the housefly possesses an enzyme known as DDT-dehydrochlorinase which metabolises DDT to DDE, a non-toxic form of the insecticide. This enzyme was later purified and subsequently demonstrated to be a GST (Clark and Shamaan, 1984).

The cell has evolved several strategies to metabolise xenobiotics. The cell is capable of inducing numerous enzymes to catalyse many different reactions on xenobiotics. Not only does the cell synthesise many different enzymes, it is also able to synthesise many different forms of the same enzyme. Currently, there are at least twelve different forms of GST in the rat liver. The number varies amongst species although the basic structure of the individual enzyme is similar for most of the species. Having multiple copies of the same enzyme enables the cell to deal with many different types of xenobiotics and also to have versatility in action on xenobiotics. Combining several different forms of the GSTs, a housefly strain can metabolise several different insecticides at the same time.

In the housefly, several types of GSTs have been documented which metabolises many different types of insecticides. A particular type of GST may react upon one particular insecticide. Having several types of GSTs may enable the housefly to react against several different types of insecticides. For example, resistant strains of the housefly contain different combinations of GSTs that can metabolise different combinations of insecticides (Clark *et al,* 1984; 1986). These findings demonstrate the versatility houseflies have in adapting to the

different insecticide stresses they have to endure in order to survive (Figure 8).
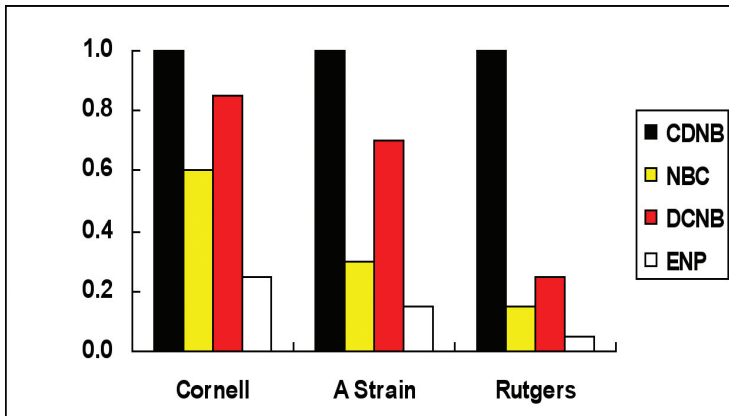
Figure 8   Activities of a similar type of GST against four different substrates in three different housefly strains collected by Cornell University, Victoria University of Wellington (A strain) and Rutgers University (adapted from Clark *et al,* 1984)

The rapid metabolism of insecticides in the housefly highlights the problems of insect control. Insects may become resistant to the insecticide in just a few generations. Thus, new and different insecticides need to be invented. This includes discovering pesticides of plant and microbial origins as well as biological control using insect predators such as the ladybird beetle which feed on aphids, bacteria such as *Bacillus thurigiensis* which produces a toxin lethal to mosquito larvae or viruses that infect insect larvae. Alternatively, different approaches are required such as proper application of pesticides, educating users on the safety aspects of pesticides, and introduction of detoxication enzyme inhibitors such as piperonyl butoxide to counteract the rapid metabolism of the insecticides by the insects. Currently, procedures for the proper usage and application of pesticides are enforced by the relevant authorities and the

pesticide industry makes it a standard procedure to add enzyme inhibitors to the formulations to increase the effectiveness of the insecticides.

Yet, with all the technology at the disposal of mankind, insects will continue to survive. There is another factor favouring the survival of insects. Many insecticides were reported to be inducers of detoxication enzymes. Thus, enzymes such as the GSTs will increase in insects that survive initial exposure to insecticides (Hayaoka and Dauterman, 1982). The surviving insect will then develop an increased ability to metabolise the insecticides leading to a decrease in the effectiveness of the insecticide.

A study was carried out to correlate insecticide toxicity to levels of detoxication enzymes in the mosquito Aedes aegypti in Klang and Kuala Lumpur (Shamaan *et al,* 1993). The results showed that there was a strong correlation between detoxication enzyme levels and insecticide toxicity in wild population of the mosquitoes in Klang and Kuala Lumpur. Different insecticide strategies need to be applied for effective control of the Aedes mosquito whereby the insecticides abate and carbaryl was found to be more effective on the Aedes mosquito larva in Kuala Lumpur than those in Klang. The level of GST activities in the Kuala Lumpur wild population was lower than that of Klang, leading to the suggestion that the Kuala Lumpur strain was more susceptible to the insecticides applied than the Klang strain.

DETOXICATION ENZYMES AND CARCINOGENESIS

Xenobiotics may also act as inducers of detoxication enzymes in the cell. This has been shown by the ability of insects to up-regulate detoxication enzyme syntheses upon exposure to insecticides. As a result, resistant insects contain higher levels of detoxication enzyme activities than those which are susceptible.

In animals, exposure to xenobiotics has resulted in the induction of detoxication enzymes. The drug phenobarbital is a well known inducer of many enzymes in the liver. Other compounds such as benzopyrene and the nitrosamines are good examples of xenobiotics acting as inducers of detoxication enzymes. In the mouse liver (Figure 9), benzopyrene was found to induce glutathione S-transferase and glutathione peroxidase activities over time (Hanachi *et al,* 2003). After four weeks of treatment with benzopyrene, tumour nodules were observed in the liver. The high levels of enzyme activities corresponded with the increased state of carcinogenesis. Microscopic examination of the liver slices showed that the liver cells have lost their natural morphology and had turned into neoplastic cells. Subsequently, it was also found that benzopyrene induced a different form of glutathione S-transferase which was larger than normal (Hanachi *et al,* 2004a; 2004b).

In the African catfish *Clarias gariepinus,* glutathione S-transferase, glutathione peroxidase and cytochrome P450 monooxygenase activities were temporarily increased after exposure to a sub-lethal concentration of the carbamate insecticide carbofuran (Daryani *et al,* 2004). The increase in enzyme activities probably reflected the ability of the fish to rapidly metabolise the insecticide in its efforts to detoxicate it.

Cancer is a disease where damaged cells do not undergo apoptosis. This means that the damaged cells will continue to grow but as mutants, very different from the original normal cell. The growth of the damaged cells will no longer be under normal control and the metabolic processes may be altered. In simple terms, cancerous cells are not controlled but are free to proliferate independent of the cells around them. Cancer is a highly complex, multifactorial disease caused partly by metabolic or other imbalances associated with age or genetic makeup and, partly by a wide variety of external factors including diet, lifestyle, ionizing radiation and xenobiotics.
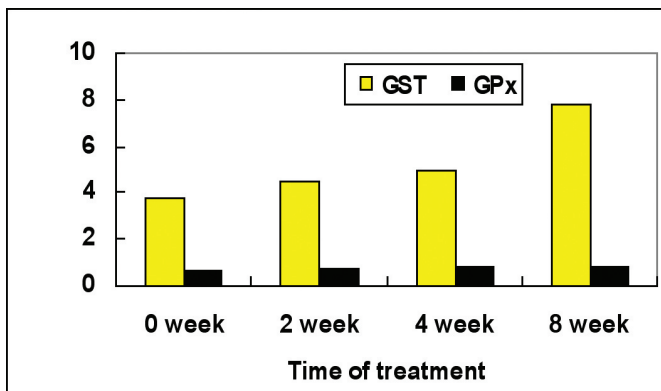
Figure 9 Effect of benzopyrene on glutathione S-transferase and glutathione peroxidase activities in the mouse.

CARCINOGEN

The term carcinogen refers to any substance, including radioactive substances and other substances that emit ionising radiation, directly involved in the promotion of cancer or which facilitates the propagation of cancer. This may be due to the ability of the carcinogen to damage the DNA of the cell or disrupt cellular metabolic processes but does not cause cell death. Common examples of carcinogens are nitrosamines, aminofluorenes, certain dioxins, cigarette or tobacco smoke, inhaled asbestos and radioactive compounds that emit ionising radiation. There are also many natural substances that are carcinogenic. Aflatoxin B_1 produced by the fungus *Aspergillus flavus* growing on stored grains, nuts and peanut butter, is a powerful naturally-occurring carcinogen. The Hepatitis B and human papilloma viruses have been found to cause cancer in humans. The first virus discovered to cause cancer in animals is the Rous sarcoma virus, discovered in 1910 by Peyton Rous.

There are a number of enzymes involved in carcinogenesis. These enzymes participate in normal metabolism of the cell but are are also so significantly

expressed in cancer that they are considered as marker enzymes. Examples of marker enzymes are the glutathione S-transferases (GST), sulphotransferase, cytochrome P$_{450}$ oxygenases, gamma-glutamyltranspeptidase (GGT), alkaline phosphatases and uridyldiphosphate glucuronyltransferase (UDPGT). These enzymes are also markers for other diseases and as such, should be used with other tests for complete diagnosis.

CARCINOGENESIS

Cancer development or carcinogenesis is a multi-step process. There are three stages in chemical carcinogenesis: initiation, promotion and progression.

Initiation of Carcinogenesis

Initiation describes the process where a carcinogen or other agents damage the DNA of the cell, alters the genetic make-up of the cell and confers on the transformed cell the potential for growth. The transformed cell continues to grow and proliferate without control by the original starting cell. This usually occurs after the carcinogen causes damage to the chromosomes and DNA, or complexes with DNA to form adducts or, the carcinogen binds to specific proteins involved in gene expression or control. As a result, there may be irreversible alteration to the control of gene expression, cell replication, growth and proliferation. The affected cells will then divide and proliferate into a population of neoplastic cells. The initiation stage is summarized in Figure 10.
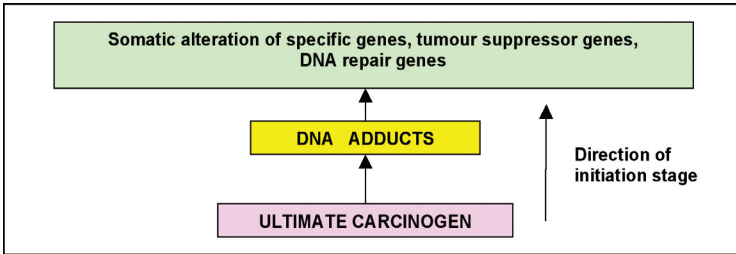
Figure 10 The initiation stage of chemical carcinogenesis

Promotion of Carcinogenesis

Promotion refers to the subsequent development and proliferation of a transformed cell through a variety of pathological states e.g. hyperplasia and neoplasia. Gene expression and cell proliferation is altered and the initiated cell is transformed into a discernible population of cancer cells. The promotion stage is illustrated in Figure 11.

In both the initiation and promotion stages, the transformed cell may revert to the original normal cell through mechanisms that restore normal processes such as DNA repair and cell de-differentiation i.e. instead of the transformed cell continuing to develop into a cancer cell, it reverts to its original normal form. Another possibility that may arise is that the cell may direct itself to apoptosis. In this case, carcinogenesis will fail to occur.
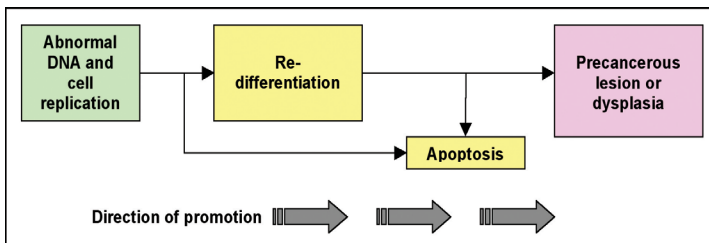
Figure 11 The promotion stage in chemical carcinogenesis

Progression of Carcinogenesis

Progression describes the events leading eventually to a malignant tumor (Farber, 1984; Office of Science and Technology Policy, 1985). The growth and expansion of the initiated and promoted cells are enhanced and accompanied by an abnormal complement of genetic material. DNA damage is widespread with loss, breakage and duplication of multiple chromosomes. The promoted cells, as a population, will progress on to metastases during which the cells will invade other tissues and translocate to other parts of the body. At this stage, several locations of the body will develop cancerous tissues at the same time. At this stage, cancer is at an advanced stage. Figure 12 illustrates the progression stage of chemical carcinogenesis.
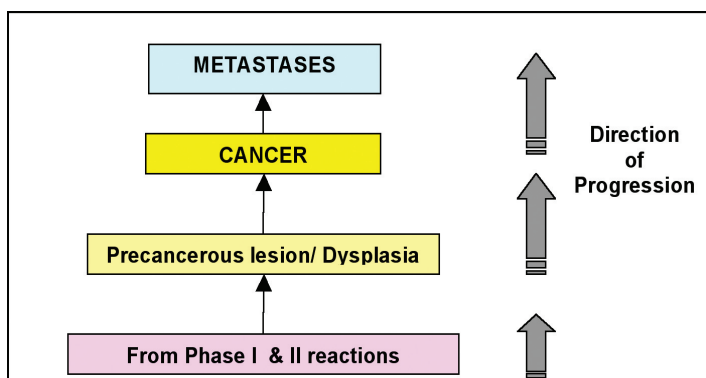
Figure 12 Progression stage in chemical carcinogenesis.

In the initiation and promotion stages of chemical carcinogenesis each consists of several stages and may involve distinct mechanisms. Some of these stages may be reversible. Probably, all the stages are susceptible to a variety of modulating factors that may show either enhancement or inhibition of the stages. For example, initiation of carcinogenesis can result directly from the mutagenic effect of a xenobiotic or its metabolite on the cell genome. Initiation

can also be an indirect result of chronic cell toxicity by the xenobiotic leading to disturbance in vital processes such as cell turnover, errors in cell replication, activation of oncogenes or other mechanisms. Initiation of carcinogenesis can also be modulated by factors that alter the efficiency of DNA repair, immune surveillance and, in the case of chemicals that require metabolic activation, factors that modify metabolism of the cell.

The detoxication enzymes play a vital role during the initiation and promotion stages of chemical carcinogenesis. Upon exposure to the xenobiotics, the Phase I and II reactions will immediately occur; firstly to biotransform the xenobiotics and secondly, to ensure that the xenobiotic metabolites are isolated and excreted from the cell. However, when detoxication mechanisms are lacking, the adverse effects of the xenobiotic will be manifested.

The chemical compounds 2-acetylaminofluorene (2-AAF) and diethylnitrosamine (DEN) are among the most potent carcinogens of liver cancer. When administered to rats, both 2-AAF and DEN induced cancer in the liver within eight weeks of administration. Light microscope examination of the liver sections revealed the formation of foci and extensive granulations in the carcinogen-treated cells compared with the control cells (Figure 13).

The activity of the detoxication enzyme glutathione S-transferase (GST) was shown to be significantly higher in the carcinogen-treated rat liver than in the control rat (Figure 14). GST activity continued to increase over the treatment period, corresponding to the increase in the severity of the chemically induced carcinogenesis (Makpol *et al,* 1996). Other tumour marker enzymes were also induced and followed similar patterns as the GST. In primary hepatocytes prepared from carcinogen-treated rats, GST and GGT activities were significantly higher than normal hepatocytes (Ong *et al,* 1994a).
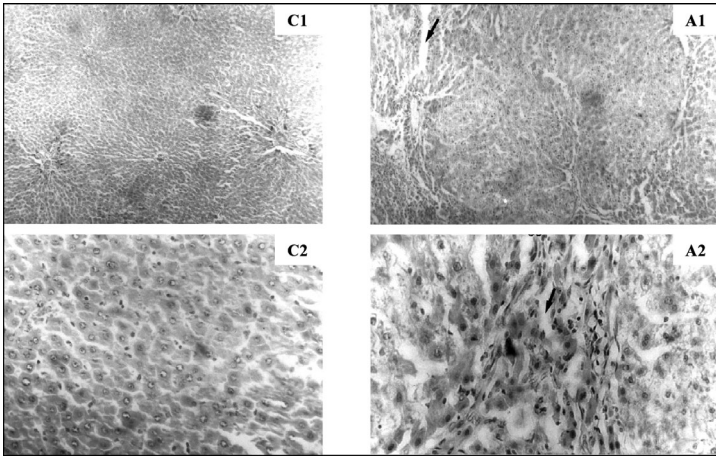
Figure 13 Stained liver sections of control and 2-AAF-treated rats.
Rat liver sections were stained with Haematoxylin and Eosin, and viewed under light
microscope. Formation of preneoplastic foci (A1) and extensive granulation (A2) is
evident in the carcinogen-treated rat liver sections. C1 and C2 are the control rat's liver
cell sections; A1 and A2 are 2-AAF-treated rat liver sections. Magnification: X10 (C1
and A1); X40 (C2 and A2)
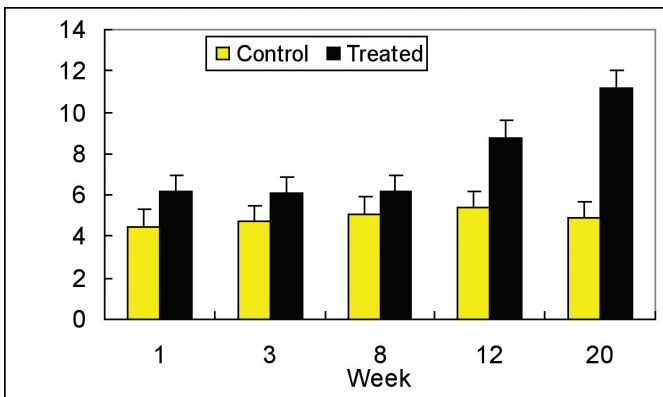
Figure 14 GST activity in the liver of control and 2-AAF-treated rats

We then investigated more closely the chemically induced liver carcinogenesis in the rat treated with the carcinogen and tocotrienols, one of the isomers of vitamin E (Wan Ngah *et al,* 1991a). Ultrastructural study of the liver revealed a change in the cellular structure and morphology, elevated levels of detoxication enzyme activities, and evidence that suggested that the tocotrienols were moderating the induction of carcinogenesis in the rat liver (Figure 15). The Control cell had its histology intact while the 2-AAF-treated cell was damaged. The shape of the nucleus in the 2-AAF cell was distorted into a horn shape instead of smooth and round. The nuclear membrane and membrane of other organelles in the 2-AAF cell were not obvious when compared with the control cell. However, there was significantly less damage to cellular integrity and structure in the 2-AAF-treated tocotrienol-supplemented cell. This observation suggested to us that tocotrienols were able to reduce the severity of carcinogenesis in rat liver. The change was not noticeable under light microscope but was obvious under electron microscopy.

Tumour marker enzyme activities were also measured in the rat livers. Plasma and liver microsomal gamma-glutamyltranspeptidase (GGT) activities were significantly higher in the carcinogen-treated rat compared with the carcinogen-treated rat which had been supplemented with tocotrienol and the control rat. The activity of another marker enzyme, uridyldiphosphate glucuronyltransferase (UDPGT) showed similar results. It was therefore concluded that tocotrienols are capable of reducing the severity of carcinogenesis in rats.

In humans, plasma GGT, glutathione peroxidase, glutathione reductase and GST activities were found to be significantly increased in patients diagnosed with nasopharyngeal carcinoma (Wan Ngah *et al,* 1993). Patients with very high GGT activities had poor prognoses but patients with decreased GGT activities were found to be in remission.
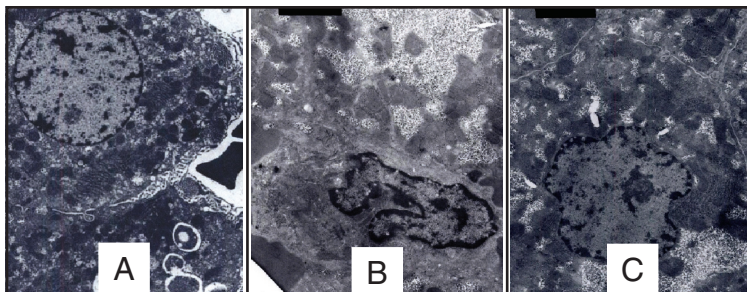
Figure 15 Electron micrograph of rat liver sections stained with hematoxylin and eosin.
Control (x 3450 magnification), A; 2-AAF-treatment (x9000 magnification), B; combined 2-AAF and tocotrienols treatment (x9000 magnification), C. While control cells show intact organelle structure with clear cell membrane and smooth round nucleus, the 2-AAF-treated cells showed cell membrane damage and severely distorted nucleus (horn-shaped). In the 2-AAF-treated cells supplemented with tocotrienols, damage was less extensive with the cell membrane still visible and the nucleus not completely deformed. (adapted from Wan Ngah *et al* (1991a), American Journal of Clinical Nutrition, 59: 1076s-1081s)

The results of other tests that we carried out also revealed that detoxication enzyme activities were closely linked to induced chemical carcinogenesis and smoking. Gamma-glutamyltranspeptidase (GGT), which is involved in the formation of mercapturic acid, was found to be increased in the liver after induction of carcinogenesis by 2-AAF in the rat (Wan Ngah et al, 1988). It was also found that GGT activity was also increased in the stomach after 2-AAF administration (Wan Ngah *et al,* 1989). It was reported that cigarette smoking alone accounted for about 30% of all male cancer cases in the United States (Doll and Peto, 1981; Higginson and Muir, 1979; Higginson, 1983).

One of the major components of cigarette smoke is nicotine, which co-incidentally, also causes addiction to smoking. Thus, in another test, our group treated the rat with nicotine and assayed for detoxication enzyme activities in

the liver (Wan Ngah *et al*, 1991b). After 100 days of treatment, there were no difference in body weight, liver and lung size between the nicotine-treated rat and control rat. However, detoxication enzyme activities (GST, UDPGT, sulphotransferase, glutathione peroxidase and glutathione reductase) were significantly increased in the nicotine-treated rat compared with the control rat. It was obvious that the nicotine treatment did not cause any adverse effects to organ size and morphology but at the molecular level there was an increase in detoxication enzyme activities, suggesting that biological processes were affected. The activities of these enzymes were sensitive to nicotine exposure.

REACTIVE OXYGEN SPECIES

In chemically induced carcinogenesis, cell damage has been attributed to oxidative stress, a condition in which oxidation reactions occur at a higher level than reduction reactions (Waris and Ahsan, 2006). This means that many components in the cell are being oxidized i.e. reacted upon towards catabolism; a metabolic process leading to degradation of the components involved. Overall, the cell will be in an exhausted state. On the other hand, a cell not under oxidative stress will be in a reduced state i.e. its anabolic rate is higher, meaning the cell is moving towards biosynthesis; a re-building state.

Oxidative stress is the result of the action of reactive oxygen species (ROS) which are formed by a variety of reactions occurring in the cell (Figure 16). Reaction oxygen species are very reactive molecules containing oxygen atoms. One example of ROS is lipid peroxides formed during the oxidation of polyunsaturated fatty acids resulting in the lipid becoming rancid.
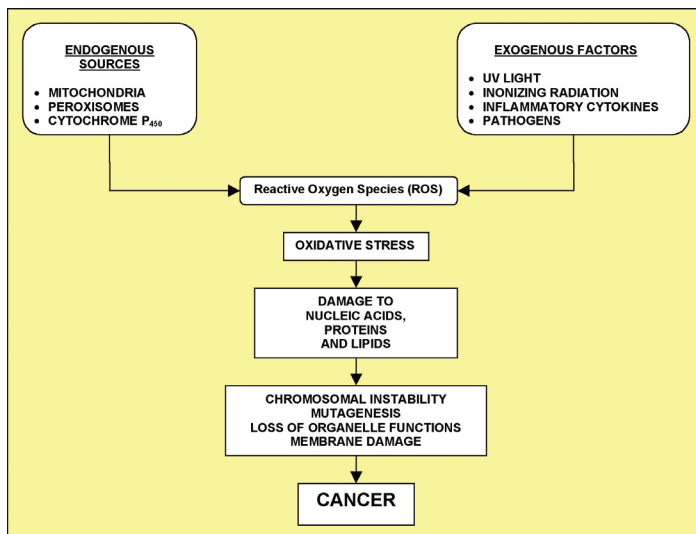
Figure 16 Role of reactive oxygen species in carcinogenesis

The lipid peroxides will start a chain reaction producing free radicals and finally resulting in the breakdown of fatty acid to smaller products. These reactions occur in sub-cellular components of the cell especially the mitochondria, peroxisomes and cytochrome P_{450} where most of the reactions involving oxygen and electrons are found. The cellular sources of free radicals arising from oxidative stress are summarized in Figure 17.

Oxidative stress can also arise due to the action of external factors such as ultra-violet radiation, inflammatory cytokines, pathogens and ionizing radiation, especially exposure to radioactive chemicals and X-rays. Molecules such as lipids, nucleic acids and proteins are reacted upon to generate free radicals which will then start chain reactions to produce ROS.
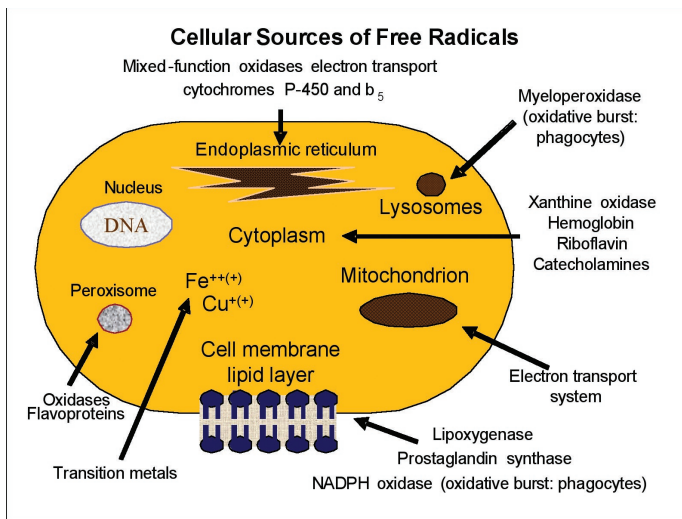
Figure 17   Cellular sources of free radicals.
Many reactions occurring naturally in the cell contribute to the generation
of free radicals.

The ROS will subsequently react with cellular components such as DNA, chromosomes and membrane resulting in cell damage or apoptosis and cancer. Understanding the role of ROS in carcinogenesis may provide avenues for prevention of carcinogenesis and possibly cancer therapy. Since the cell undergoes oxidative stress, it may be possible to alleviate it using several methods, such as curbing smoking habits, exercising regularly, and eating more fresh fruits and vegetables. Avoiding exposure to ultra-violet and ionizing radiation can alleviate oxidative stress. A good example is using sunblock during the summer months or spending less time in the sun to prevent sunburns.

ANTIOXIDANTS

Any substance that reduces oxidative damage caused by free radicals or ROS is called an antioxidant. Free radicals are highly reactive chemicals that attack molecules by capturing electrons and modifying the chemical structures of the molecules under attack. An example of free radical reaction would be the breakdown of fatty acids into two short chain fatty acids which will in turn disturb the structure of a membrane.

Antioxidants include a number of enzymes and other substances such as vitamin C, vitamin E and many plant compounds that are capable of counteracting the damaging effects of oxidation. Antioxidants are also commonly added to products like vegetable oils and prepared foods to prevent or delay their deterioration due to the action of air. Antioxidants may possibly reduce the risks of cancer and age-related diseases (Omenn *et al,* 1994).

Tropical fruits are rich in antioxidants

Antioxidants and Prevention of Carcinogenesis

Antioxidants neutralize free radicals generated as by-products of normal cell processes. Exposure to various environmental factors including tobacco smoke and radiation, can also lead to free radical formation. Antioxidants are often described as scavengers of free radicals, meaning they neutralize the electrical charge of the free radicals and prevent them from taking electrons from other molecules. By neutralizing the action of free radicals in oxidation reactions of

molecules such as fatty acids and DNA, antioxidants help preserve the normal function and structure of the cell.

Antioxidants have been reported to play a major role in the prevention of cancer (Hennekens et al 1996; Lee *et al,* 1999).

Sources of Antioxidants

Natural antioxidants are abundant in fruits and vegetables, nuts, grains and some meats, poultry and fish. These food sources contain chemical compounds that display antioxidant properties (Table 1);

Table 1 Types of antioxidants and their food sources

Antioxidant	Food Sources
Beta-carotene	Naturally colourful foods including sweet potatoes, carrots, squash, apricots, pumpkin, mangos and some green leafy vegetables e.g. spinach
Lutein	Green, leafy vegetables
Lycopene	Fresh fruits e.g. tomatoes, watermelon, guava, papaya, grapefruit, oranges and other foods.
Selenium (mineral)	Plant foods especially rice and wheat, fresh meat and nuts.
Vitamin A	Foods rich in vitamin A include liver, sweet potatoes, carrots, milk, egg yolks and dairy products.
Vitamin C	Many fruits, vegetables, cereals, beef, poultry and fish.
Vitamin E (alpha-tocopherol)	Found in almonds, in many oils including wheat germ, safflower, corn, palm and soybean oils, and in mangos, nuts, broccoli and other foods.

Cells also contains many substances that may function as antioxidants. These may include vitamin E, vitamin C, glutathione and glutathione-dependent enzymes and other enzymes namely the peroxidases and reductases. These molecules and enzymes function to prevent oxidation of biomolecules such as fatty acids, nucleic acids and proteins.

In induced chemical hepatocarcinogenesis in the rat, supplementation of antioxidants namely vitamin C and *Aloe vera* extracts were found to protect against chemically induced hepatocarcinogenesis in the rat (Shamaan *et al,* 1998a). Tumour marker enzyme activities especially GGT, UDPGT, plasma alkaline phosphatase and placental GST, were markedly increased in the liver of the carcinogen-treated rat. When vitamin C and *Aloe vera* extract were administered singly or in combination to the carcinogen-treated rat, increase in tumour marker enzyme activities were moderated. In addition, immunohistochemical analysis of the extent of enzyme-positive foci formed in the liver cell revealed that both vitamin C and *Aloe vera* extract reduced the rate of pre-neoplastic change, providing additional evidence that these antioxidants were able to reduce carcinogenesis in the liver. Vitamin C is reported to be the main ingredient of the Aloe vera extract and thus, it displayed similar properties in the mode of action in reducing the severity of carcinogenesis.

Aloe vera plants from which gel extracts are manufactured.

The mushroom *Ganoderma tsugae* Murr. (Aphyllophoromycetideae), more commonly known as Lingzhi mushroom, have been known to display anticancer properties (Yun *et al,* 1995). When it was fed to normal rats and rats treated with the carcinogen diethylnitrosamine (DEN) and 2-acetylaminofluorene (2-AAF), the Ganoderma extract reduced the number of enzyme-positive foci and attenuated the induction of tumour marker enzymes in the liver (Shamaan *et al,* 2000c). The results obtained using Ganoderma were found to be similar to that of using vitamin E; where an increase in tumour marker enzyme activities was moderated. Although supplementation of ganoderma either before or after initiation of cancer did not show any difference in effect; it did reduce the severity of cancer.

Ganoderma spp. growing in the wild.

Comparing the action of vitamin E and beta-carotene on chemically induced hepatocarcinogenesis in the rat, it was found that beta-carotene reduced the severity of cancer (Shamaan *et al,* 1997). The action of beta-carotene is similar to that of vitamin E and there was no synergistic effect when beta-carotene was combined with vitamin E in supplementation. The combined supplementation of three antioxidants, vitamin C, vitamin E and *Aloe vera* extract also did not show any synergistic effect in reducing the severity of induced chemical

hepatocarcinogenesis (Shamaan *et al,* 1998b). The combined supplementation of these three antioxidants protects from hepatocarcinogenesis.

It can be seen that antioxidants such as vitamin C, E, plant pigments such a beta-carotene, and Aloe vera and ganoderma possess chemopreventive properties against induced chemical carcinogenesis. The freshwater algae, *Chlorella vulgaris,* is rich in antioxidants and has also been proven to be chemopreventive in carcinogenesis in the rat (Sulaiman *et al,* 2006).

Chlorella vulgaris is also a potentially sensitive biological indicator monitoring the presence of herbicides especially atrazine, simazine and diuron (Shamaan *et al,* 2000a; 2000b). The photosynthetic rate of the freshwater algae was markedly inhibited by very low amounts of the herbicides (Figure 18).
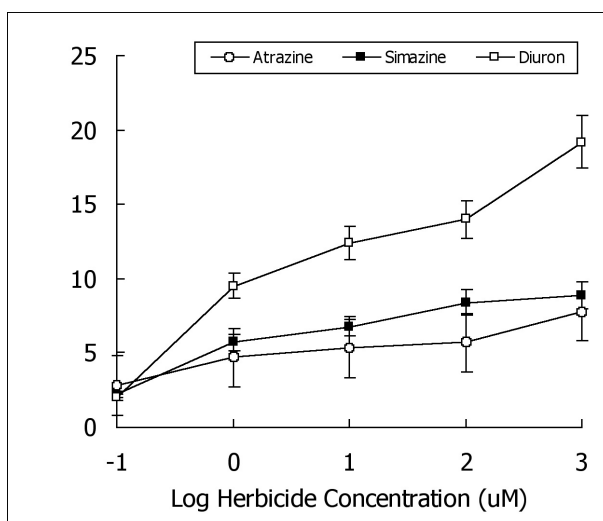

Figure 18 Relative fluorescence of *C.vulgaris* at different herbicide concentrations. Relative fluorescence is the difference between the values of treated samples and controls.

The main components in vitamin E are alpha-tocopherol and gamma-tocotrienol. Having established that vitamin E helps prevent cancer, it was later found that alpha-tocopherol was better at reducing the severity of cancer than gamma-tocotrienol (Makpol *et al,* 1997). While supplementing vitamin E at higher doses did not exhibit any additional effect; alpha-tocopherol was found to work better than gamma-tocotrienol at an optimal low dose of 30 mg per kg body weight.

In an earlier study, the optimum doses of alpha-tocopherol and gamma-tocotrienol for protection against cancer in the rat was determined at 34 mg per kg body weight and 30 mg per kg body weight, respectively (Rahmat *et al,* 1993a). This dose was subsequently used in our other studies. Supplementing vitamin E at the optimum dose in rats treated with DEN and 2-AAF over the long term resulted in a significant reduction in the severity of cancer (Rahmat *et al,* 1993b). Gamma-tocotrienol or alpha-tocopherol on its own had no effect on tumour marker enzyme activities (Shamaan et al, 1993). Its effect is only obvious in induced chemical hepatocarcinogenesis.

During cancer, the immune response of the body is greatly reduced. The body becomes weak and more susceptible to infections. Supplementing vitamin E to rats treated with carcinogens significantly enhanced their immune response where vitamin E showed a dose-dependent trend with mitogenesis and phagocytic activity of peritoneal macrophages (Ong *et al,* 1994b).

In cultured rat hepatocytes prepared from normal and carcinogen-treated rats, alpha-tocopherol showed different properties compared with gamma-tocotrienol (Ong *et al,* 1993). Alpha-tocopherol was more effective in reducing GGT activity at a higher dose in the cultured hepatocytes of the carcinogen-treated rat liver while gamma-tocotrienol was more effective at low doses. These findings suggest that it may be possible to reduce severity of cancer by combining the low gamma-tocotrienol dose and the high alpha-tocopherol dose in vitamin E supplements.

Synthetic hormones may also display anticancer activity. Danazol and clomiphene are both synthetic drugs used in treating hormone disorders. Danazol is used in the treatment of endometriosis while clomiphene is used to treat women suffering from amenorrhea. Recent studies have shown that danazol and clomiphene are cytotoxic to cancer cells. By monitoring tumour marker enzyme activities, cell proliferation and viability, and immunohistochemical analysis, our group found that both danazol and clomiphene were able to protect against cancer in the rat, both *in vivo* and *in vitro*, respectively (Shamaan *et al,* 2000d).

APPLICATIONS OF DETOXICATION ENZYMES

Thus far, knowledge of the ability of living organisms to metabolise xenobiotics has two major implications. Firstly, since detoxication enzymes are induced by xenobiotics, there is potential to develop these enzymes as indicators of the presence of xenobiotics. The glutathione S-transferases are regularly monitored in insect populations for resistance to insecticides, and in humans, for diagnoses of certain types of cancer. Plasma GGT, glutathione peroxidase, alkaline phosphatases and the cytochrome dependent enzymes are established markers for certain diseases while specific types of the glutathione S-transferases are now established as markers for certain types of cancer. Acetylcholinesterase is another detoxication enzyme that has potential as a biosensor for application in detection of pollutants. It is sensitive to xenobiotics, especially some heavy metals.

Secondly, the ability to metabolise xenobiotics is a major factor to be considered in designing new drugs. Resistance to insecticides in insects and antibiotics resistance shown by bacteria has influenced the design of new insecticides and drugs, respectively. In man and animals, the metabolic fate of drugs and medicine needs to be detailed out before it can be considered for use. With increased knowledge of xenobiotic metabolism in bacteria and other microorganisms, bioremediation of the environment is becoming an important

option for the country in maintaining a safe environment.

The Role of Microbes in Detoxication - Bioremediation

Bioremediation can be defined as any process that uses microorganisms or their enzymes, to restore the natural environment that has been altered by contaminants to its original condition. Microorganisms, especially bacteria, have been successfully employed to degrade specific soil contaminants such as chlorinated hydrocarbons. Bacteria is known to be able to degrade almost anything it comes across. From a microbiologist's point of view, the bacteria seems to have a policy of "if you can't beat them, eat them".

Microbial Degradation

As mankind tries to find new, more efficient and environmental friendly ways to clean contaminated environments, microbial biodegradation of pollutants seems to be receiving greater attention. The ability of microbes to degrade, transform or bioaccumulate a wide range of xenobiotics, including oil, chlorinated hydrocarbons, drugs and pharmaceuticals, and heavy metals, makes it desirable to be used in bioremediation on a sustainable basis. Current and new technologies such as DNA technology, protein chemistry, imaging technology, bioinformatics and high throughput analyses have made it possible to obtain a detailed understanding of the bacterial genomic, proteomic and metabolic profiles of the bacteria. This information will provide detailed insights into the key biodegradative pathways which form the basis for the versatility and adaptability of bacteria to survive in a contaminated and hostile environment

Biological processes play a major role in the removal of contaminants. Microorganisms are able to degrade and convert contaminants into harmless products. With in depth knowledge of the biochemical pathways and current technologies, it is now possible to track the metabolic fate of a given xenobiotic in a particular environment and at the same time, be able to identify the type

of microbes able to degrade it. This knowledge will certainly accelerate the development of bioremediation technologies and biotransformation processes (Diaz, 2008). A good example of such a biological process is demonstrated in the clean up of oil spills. Bioremediation of oil spills was enhanced when nitrate and sulphate fertilizers were added into the sites. The bacteria used in the biodegradation process utilised both nitrates and sulphates as sources for growth and proliferation.

It should be noted that not all contaminants can be easily biodegraded by microorganisms. Heavy metals such as lead, cadmium and mercury are not readily taken up by microorganisms. As such, these heavy metals may persist in the environment in their stable state and the risk of them being absorbed into the food chain is high. Mercury, for example, entered the food chain and caused Minamata's disease in humans consuming contaminated fish from Minamata Bay in the 1960s. Certainly, for heavy metals, one obvious way for clean up is by using microorganisms or plants (including transgenic varieties) that can absorb and bioaccumulate heavy metals in their system. Harvesting the plants may remove heavy metals from the environment (Meagher, 2000). For bioremediation of radioactive metals, the transgenic bacteria, *Deinococcus radiodurans*, the most radioresistant organism known, has been modified to consume and digest toluene and mercury ions from nuclear wastes (Brim *et al,* 2000).

Molybdenum is an element found in the soil mainly in the form of molybdate. In industry, it is often used in high strength steel super alloys and nickel base alloys due to its very high melting temperature of about $2600^{\circ}C$, in lubricants, chemicals, glass workings, ink, pigments and electronics, and many other applications. According to the Department of Environment, Malaysia, it is from the scheduled wastes from these kinds of industries that molybdenum can be found in the wastes generated in Malaysia. A significant portion of these scheduled wastes have been found to be illegally discharged or dumped and have caused several of the pollution cases reported in the country.

In the biota, molybdenum is found in trace amounts in plants and animals as important components of the oxidases involved in protein synthesis, nucleic acid synthesis, nitrogen-, carbon- and sulphur-cycles. In soil bacteria, trace amount of molybdenum is found in the nitrogen fixation enzymes. Although molybdenum in trace amounts is a vital component of enzymes, it can be inhibitory in excessive amounts whereby it can inhibit growth and other metabolic processes such as protein and nucleic acid synthesis.
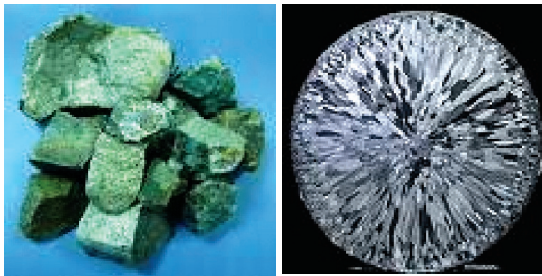
Molybdenum – gray-metallic element used in high strength steel alloy
(from Wikipedia)

Isolation of molybdenum-reducing bacterium for the purpose of bioremediation of molybdenum is very important in dealing with the emerging pollution problems caused by molybdenum. Molybdate reduction was first reported in *E. coli* in 1986, followed by other bacteria such as *Bacillus typhi (Salmonella typhi), Serratia sp., Micrococcus lactyliticus, Pichia guilliermondii* and *Micrococcus sp., E. Coli* K12, *Thiobacillus ferrooxidans* and *Enterobacter cloacae* strain 48 (EC 48) (Shukor *et al.*, 2000). The reduction of molybdate into molybdenum blue by the *Thiobacillus ferrooxidans* strain AP19-3, a chemolitotroph, was initially attributed to the enzyme sulphur:ferric ion oxidoreductase (SFORase), but was later reported to be due to the action of a molybdate reducing enzyme (Shukor *et al.,* 2000). Subsequently, it was proven that molybdate reduction in the *Enterobacter cloacae* strain 48 is enzymatic (Shukor *et al.,* 2002;2003). We

also found that molydophosphate is an important intermediary (Shukor *et al.,* 2007) in the reaction and that arsenate and phosphate inhibitions of molybdate reduction were not at the enzymatic level (Shukor *et al.,* 2008a). Recently, we have isolated and characterized the molybdenum-reducing *Serratia marcescens* strain Dr. Y6 bacterium from Malaysian soils (Shukor *et al.,* 2008b). The enzyme catalysed molybdenum reduction is represented in Figure 19 shown below.
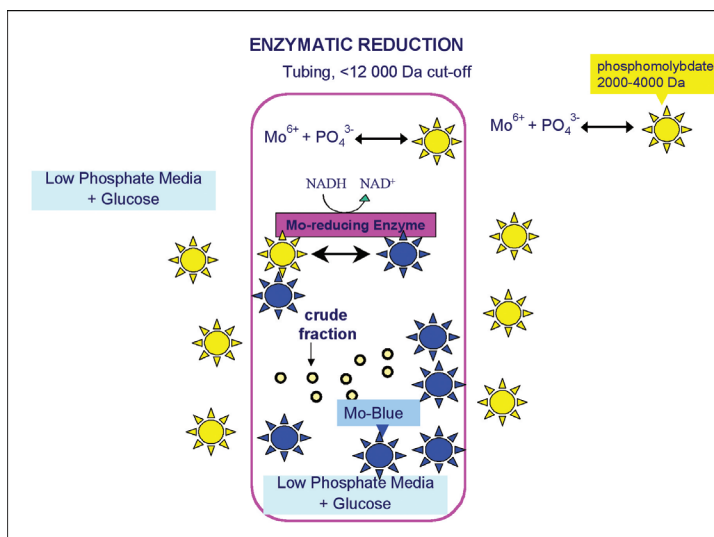
Figure 19 Enzymatic reduction of molybdate to Mo-blue. Yellow and blue stars denote unreduced and reduced molybdophosphates, respectively.

CONCLUSIONS

Living organisms have the ability to metabolise any foreign compounds for its survival and to do this, enzymes play an important role in catalyzing the reactions involving the foreign compounds. Metabolism of foreign compounds, especially drugs and other substances, is an important factor to be considered during drug design and discovery. Knowledge of the metabolism of these compounds

can be utilized towards maintaining a healthy body by applying concepts and principles of the biochemical pathways involved. These same principles are also utilized towards maintaining a safe environment with bioremediation as a potentially useful tool.

REFERENCES

Abu Zeid I.M., Syed M.A., Ramli J., Arshad J.H., Omar I. and Shamaan N.A. 2004. Amino acid transaminases and acetylcholinesterase activities in the African catfish, *Clarias gariepinus* and their susceptibility to exposure of sub-lethal concentrations of carbofuran and endosulfan. *Pertanika Journal of Science and Technology* **12(2)**: 225-233

Boyland E., and Chasseaud L.F. 1969. The role of glutathione and glutathione S-transferases in mercapturic acid biosynthesis. *Advances in Enzymology and Related Areas in Molecular Biology* **32**: 173–219.

Brim H., McFarlan S.C., Fredrickson J.K., Minton K.W., Zhai M., Wackett L.P. and Daly M.J. 2000. Engineering Deinococcus radiodurans for metal remediation in radioactive mixed waste environments. *Nature Biotechnology* **18(1)**: 85 – 90.

Clark A.G., and Shamaan N.A.1984. Evidence that DDT dehydrochlorinase from the housefly is a glutathione S-transferase. P*esticide Biochemistry and Physiology* **22**: 249-261

Clark A.G., Shamaan N.A., Dauterman W.C. and Hayaoka T. 1984. Characterization of multiple glutathione S-transferases from the housefly, Musca domestica (L). *Pesticide Biochemistry and Physiology* **22**: 51-59

Clark A.G., Shamaan N.A., Sinclair M.D. and Dauterman W.C. 1986. Insecticide metabolism by multiple glutathione S-transferases in two strains of the housefly, *Musca domestica. Pesticide Biochemistry and Physiology* **25**: 169-175

Commandeur J.N., Stijntjes G.J., Vermeulen N.P. 1995. Enzymes and transport systems involved in the formation and disposition of glutathione S-conjugates. Role in bioactivation and detoxication mechanisms of xenobiotics. *Pharmacological Reviews* **47(2)**: 271–330.

Daryani, Syed M.A., Arshad J.H., Ramli J., and Shamaan N.A. 2004. Toxicity and effect of carbofuran on enzyme activities in the African catfish *Clarias gariepinus. Malaysian Journal of Biochemistry and Molecular Biology* **9**: 16-21

Diaz E (editor). 2008. Microbial Biodegradation: Genomics and Molecular Biology, 1st ed., Caister Academic Press.

Doll, R.; Peto, R. The Causes of Cancer; Oxford University Press: New York, 1981.

Farber, E. 1984 . Cellular biochemistry of the stepwise development of cancer with chemicals: G. H. A. Clowes memorial lecture. *Cancer Research* **44**: 5463–5474.

Guengerich F.P. 2001. Common and uncommon cytochrome P_{450} reactions related to metabolism and chemical toxicity. *Chemical Research in Toxicology.* **14(6)**: 611–50.

Habig W.H., Pabst M.J., Jakoby W.B. 1974. Glutathione S-transferases: The first enzymatic step in mercapturic acid synthesis. *Journal of Biological Chemistry* **249**: 7130-7139.

Hanachi P., Shamaan N.A., Ramli J., Arshad J.H., Syed M.A. 2004b. Evidence that benzo[a]pyrene induced large glutathione S-transferase subunits in the mouse *Mus musculus. Pakistan Journal of Biological Science* **7(2)**: 209-211.

Hanachi P., Shamaan N.A., Ramli J., Arshad J.H. and Syed M.A. 2003. Effect of benzo[a]pyrene on glutathione S-transferase and glutathione peroxidase activities and liver and kidney histology in white mice *Mus musculus. Pakistan Journal of Medical Sciences* **19**: 197-202.

Hanachi P., Syed M.A., Omar I., Ramli J., Arshad J.H., Yunus I. and Shamaan N.A. 2004a. Purification of liver glutathione S-transferases in benzo[a]pyrene-treated and normal mice by affinity chromatography. *Malaysian Journal of Biochemistry and Molecular Biology* **9**: 7-11.

Hayaoka T. and Dauterman W.C. 1982. Induction of glutathione S-transferases by phenobarbital and pesticides in various housefly strains and its effect on toxicity. *Pesticide Biochemistry and Physiology* **17**: 113-119.

Hennekens C.H., Buring J.E., Manson J.E., Stampfer M., Rosner B., Cook N.R., Belanger C., LaMotte F., Gaziano M., Ridker P.M., Willett W., and Peto R. 1996.

Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *New England Journal of Medicine* **334**:1145-9.

Higginson J. 1983. Developing concepts on environmental cancer: The role of geographical pathology. *Environmental Mutagenesis* **5**: 929-40.

Higginson J. and Muir C.S. 1979. Environmental carcinogenesis: Misconceptions and limitations to cancer control. *Journal of the National Cancer Institute* **63** 1291-98.

Jakoby W.B., and Ziegler D.M. 1990. The enzymes of detoxication. *Journal of Biological Chemistry* **265(34)**: 20715–8.

König J., Nies A.T., Cui Y., Leier I, and Keppler D. 1999. Conjugate export pumps of the multidrug resistance protein (MRP) family: localization, substrate specificity, and MRP2-mediated drug resistance. *Biochimica et Biophysica Acta* **1461(2)**: 377–94.

Lee I.M., Cook N.R. and Manson J.E. 1999. Beta-carotene supplementation and incidence of cancer and cardiovascular disease: Women's Health Study. *Journal of the National Cancer Institute* **91**: 2102-6.

Makpol S., Wan Ngah W.Z., Shamaan N.A., Jarien Z., Marzuki A. and Khalid B.A.K. 1996. γ-Glutamyl transpeptidase, glutathione S-transferase, alkaline phosphatase and glutathione levels during different stages of induced chemical hepatocarcinogenesis in the rat. *Journal of Clinical Biochemistry and Nutrition* **20**: 121-129.

Makpol S., Wan Ngah W.Z., Shamaan N.A., Jarien Z., Md Top A.G. and Khalid B.A.K. 1997. Different starting times of vitamin E supplementation and tumour marker enzyme activitles in the rat chemically induced with cancer. *General Pharmacology* **28**: 589-592.

Meagher, R.B. 2000. "Phytoremediation of toxic elemental and organic pollutants". *Current Opinion In Plant Biology* **3(2)**: 153-162.

Motoyama N., Hayaoka T., Nomura K., and Dauterman W.C. 1980. Multiple factors for organophosphorus resistance in the housefly, *Musca domestica* L., *Journal of Pesticide Science* **5**: 393 - 400.

Office of Science and Technology Policy. 1985 . "Chemical Carcinogens: A Review of the Science and its Associated Principles"; *Federal Registry* **50**:10371-10442.

Omenn G.S., Goodman G., Thornquist M., Grizzle J., Rosenstock L., Barnhart S., Balmes J., Cherniack M.G., Cullen M.R., Glass A., Keogh J., Meyskens Jr. F., Valanis B., Williams Jr. J. 1994. The beta-carotene and retinol efficacy trial (CARET) for chemoprevention of lung cancer in high risk populations: smokers and asbestos-exposed workers. *Cancer Research* **54(7)**: 2038s-43s.

Ong F.B., Wan Ngah W.Z., Marzuki A., Khalid B.A.K., Abdullah N., Md Top A.G., and Shamaan N.A. 1994b. Effect of Vitamin E supplementation on the immune response during chemically induced hepatocarcinogenesis in the rat. *Journal of Clinical Biochemistry and Nutrition* **17**: 161-169.

Ong F.B., Wan Ngah W.Z., Md Top A.G., Khalid B.A.K. and Shamaan N.A. 1994a. Vitamin E, glutathione S-transferase and γ-glutamyl transpeptidase activities in cultured hepatocytes of rats treated with carcinogens. *International Journal of Biochemistry* **26(3)**: 397-402.

Ong FB, Wan Ngah WZ, Shamaan NA, Md. Top AG, Marzuki A and Khalid BAK (1993). Glutathione S-transferase and γ-glutamyl transpeptidase activities in cultured rat hepatocytes treated with tocotrienol and tocopherol. *Comparative Biochemistry and Physiology* **106C**: 237-240.

Rahmat A., Wan Ngah W.Z., Marzuki A., Jarien Z., Ismail R., Khalid B.A.K. and Shamaan N.A. 1993a. Effect of γ-tocotrienol and γ-tocopherol on blood glutathione and tumour marker enzyme activities during chemical hepatocarcinogenesis in the rat. *Journal of Clinical Biochemistry and Nutrition* **15**: 195-202.

Rahmat A., Wan Ngah W.Z., Shamaan N.A., Md. Top A.G. and Khalid B.A.K. 1993b. Effect of long term administration of tocotrienols on tumour marker enzyme activities during hepatocarcinogenesis in rats. *Nutrition* **9**: 229-232.

Rogan W.J., Chen A. 2005. "Health risks and benefits of bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT)". *Lancet* **366(9487)**: 763–73.

Schlichting I., Berendzen J., Chu K., Stock A.M., Maves S.A., Benson D.E., Sweet B.M., Ringe D., Petsko G.A., Sligar S.G. 2000. The catalytic pathway of cytochrome p450cam at atomic resolution. *Science* **287(5458)**: 1615–22.

Shamaan N.A., Abu Zeid I.M., Ramli J., Arshad J.H., Omar I. and Syed M.A. 2005. Bioaccumulation of carbofuran and endosulfan in selected tissues of the African catfish, Clarias gariepinus. *Pertanika Journal of Science and Technology* **13(2)**:249-256.

Shamaan N.A., Akim M.A., Jarien Z., Ramli J., Syed M.A. and Wan Ngah W.Z. 1998b. Effect of supplementation of *Aloe vera* extract, vitamin C and in combination with vitamin E on enzyme activities in rat hepatocarcinogenesis. *Malaysian Journal of Biochemistry and Molecular Biology* **3**: 12-15.

Shamaan N.A., Desa S., Omar I., Kusnan M. and Omar H. 2000a. Freshwater algae as a biological marker. 1- The selection of *Chlorella vulgaris* as a test organism for herbicide toxicity using chlorophyll fluorescence. *Malaysian Journal of Biochemistry and Molecular Biology* **5**: 10-13.

Shamaan N.A., Desa S., Omar I., Kusnan M.. and Omar H. 2000b. Freshwater algae as a biological marker. 2- Suitability of *Chlorella vulgaris* as a test organism for the toxicity of photosystem II herbicides using chlorophyll fluorescence. *Malaysian Journal of Biochemistry and Molecular Biology* **5**: 14-17.

Shamaan NA, Jarien Z, Top A.G.M., Khalid B.A.K and Wan Ngah W.Z. 2000d. Effect of Ganoderma tsugae Murr. (Aphyllophoromycetideae) and vitamin E supplementation on enzyme activities in the liver and plasma of normal and chemically induced hepatocarcinogenic rats. *International Journal of Medicinal Mushroom* **2**: 133-139.

Shamaan N.A., Ong F.B., Jarien Z., Adam A., Marzuki A., Khalid B.A.K. and Wan Ngah W..Z (1997). Combined supplementation of β-carotene and vitamin E in chemical hepatocarcinogenesis in the rat. *Malaysian Journal of Biochemistry and Molecular Biology* **2**: 33-36.

Shamaan N.A., Reduan H., Abdul Jalil H., Jeffries J. and Wan Ngah W.Z., 1993. Insecticide toxicity, glutathione S-transferase and carboxylesterase activities in the larva of Aedes mosquito. *Comparative Biochemistry and Physiology* **104C**: 107-110

Shamaan N.A., Wan Ngah W.Z., Ibrahim R., Jarien Z., Md. Top A.G. and Khalid B.A.K. 1993. Effect of tocotrienol on the activities of cytosolic glutathione dependent enzymes in rats treated with 2-acetylaminofluorene. *Biochemical Pharmacology* **45**: 1517-1519.

Shamaan N.A., Wan Ngah W.Z., Jarien Z., Ismail R., Rahmat A., Jubri Z. and Khalid B.A.K. 1998a. Vitamin C and *Aloe vera* supplementation protects from induced chemical hepatocarcinogenesis in the rat. *Nutrition* **14**: 846-852.

Shamaan N.A., Wan Ngah W.Z., Jarien Z., Jantan I. and Khalid B.A.K. 2000c. Danazol and clomiphene reduced the severity of hepatocarcinogenesis in the rat *in vivo* and cancer cells *in vitro*. *Asia Pacific Journal of Pharmacology* **14**: 67-72.

Shukor M.Y.A., Lee C.H., Karim M.I.A., Syed M.A., and Shamaan N.A. 2002. A method to distinguish between chemical and enzymatic reduction of molybdenum in *Enterobacter cloacae* Strain 48. *Malaysian Journal of Biochemistry and Molecular Biology* **7**: 71-72.

Shukor M.Y.A., Shamaan N.A., Syed M.A., Lee C.H. and Karim M.I.A. 2000. Characterization and quantification of molybdenum blue production in *Enterobacter cloacae* Strain 48 using 12-molybdophosphate as the reference compound. *Asia Pacific Journal of Biotechnology and Molecualr Biology* **8**: 167-172

Shukor M.Y.A., Syed M.A., Lee C.H., Omar I., Karim M.I.A. and Shamaan N.A. 2003. Isolation and characterization of a molybdenum reducing enzyme in *Enterobacter cloacae* Strain 48. *Pertanika Journal of Science and Technology* **11(2)**: 261-272.

Shukor Y., Adam H., Ithnin K., Yunus I., Shamaan N.A. and Syed M.A. 2007. Molybdate reduction to Molybdenum blue in microbe proceeds via a phosphomolybdate intermediate. *Pakistan Journal of Biological Science* **7(8)**: 1448-1452.

Shukor M.Y., Habib S.H.M., Rahman M.F.A., Jirangon H., Abdullah M.P.A., Shamaan N.A. and Syed M.A. 2008a. Hexavalent molybdenum reduction to molybdenum blue by S. marcescens Strain Dr.Y6. *Applied Biochemistry and Biotechnology* DOI: 10.1007/s12010-008-8137-z.

Shukor M.Y., Shamsuddin B., Mohamad O., Ithnin K., Shamaan N.A. and Syed M.A. 2008b. A method to study the effects of chemical and biological reduction of molybdate to molybdenum blue in bacteria. *Pakistan Journal of Biological Science* **11(4)**: 672-675.

Sulaiman S., Shamaan N.A., Wan Ngah W.Z. and Mohd Yusof Y.A. 2006. Chemopreventive effect of *Chlorella vulgaris* in choline deficient diet and ethionine induced liver carcinogenesis in rats. *International Journal of Cancer Research* 2(3): 234-241.

Truman's Scientific Guide to Pest Control Operations, 5th edition, 1997. Gary Bennett, John M. Owens, and Robert M. Corrigan. Advanstar Communications, Duluth, MN.

Wan Ngah WZ, Jarien Z and Shamaan NA (1988). Effect of 2-acetylaminofluorene on γ-glutamyltranspeptidase activities in rat plasma and liver microsomes. Malaysian *Journal of Medical Laboratory Sciences* **5**: 29-32.

Wan Ngah W.Z., Jarien Z., and Shamaan N.A. 1989. Effect of 2-acetylaminofluorene on cytosolic glutathione S-transferase activities and morphology in rat liver and stomach. *Sains Malaysiana* **18(3)**: 17-25.

Wan Ngah W.Z., Jarien Z., Rajikin M.H., and Shamaan N.A. 1991b. Effects of nicotine on glutathione metabolising enzymes and some conjugation enzymes in the rat. *Asia Pacific Journal of Pharmacology* **6**: 55-61.

Wan Ngah W.Z., Jarien Z., San M.M., Marzuki A., Md. Top G., Shamaan N.A. and Khalid B.A.K. 1991a . The effects of tocotrienols on hepatocarcinogenesis induced by 2-acetylaminofluorene in rats. *American Journal of Clinical Nutrition* **53**: 1076S-1081S.

Wan Ngah W.Z., Shamaan N.A., Said M.H. and Azhar M.T. 1993. Activities of γ-glutamyl transpeptidase and erythrocyte glutathione dependent enzymes in nasopharyngeal carcinoma patients and normal controls. *European Archives of Otorhinolaryngology* **250**: 304-307

Waris G., and Ahsan H. 2006. Reactive oxygen species: role in the development of cancer and various chronic conditions. *Journal of Carcinogenesis* **5**:14 doi:10.1186/1477-3163-5-14.

Williams R.T. Detoxication mechanisms. The metabolism and detoxication of drugs, toxic substances and other organic compounds. John Wiley and Sons Inc., New York, 1959.

Yun T.K., Kim S.H. and Lee Y.S. 1995. Trial of a new medium-term model using benzo(a)pyrene induced lung tumour in newborn mice. *Anticancer Research* **15**: 839-945.

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"Gratitude unlocks the fullness of life.
It turns what we have into enough, and more.
It turns denial into acceptance, chaos to order, confusion to clarity.
It can turn a meal into a feast, a house into a home, a stranger into a friend".

I dedicate this piece of work to my late father, Haji Shamaan bin Yeop Abdullah Sani, who advised me to be in academia instead of joining the corporate world at the start of my working life, and my mother Hajah Juriah binti Osman, for persevering in instilling a bit of discipline in me and most of all for her ever continuous do'a for my success.

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"Kindness in words creates confidence.
Kindness in thinking creates profoundness.
Kindness in giving creates love".

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BIOGRAPHY

Nor Aripin Shamaan was born in Grik, Perak on 13 April 1953. He received his early education at the Methodist English School, Tanjong Malim and the Malay College Kuala Kangsar, where he is more popularly known as NorA, a nickname that has become permanent. After obtaining his A levels at Poole Technical College, Dorset, England, Nor Aripin went to the University of Liverpool and graduated with a BSc (Hons) in Biochemistry in 1976. He returned to Malaysia and joined Universiti Pertanian Malaysia as a tutor in the Department of Biochemistry and Microbiology, and then proceeded to the Victoria University of Wellington, New Zealand, in 1978 to do his PhD in Insecticide Biochemistry. Dr. Nor Aripin returned as a lecturer in 1982 and taught courses such as Protein and Nucleic Acid Metabolism, Carbohydrate Metabolism, Basic Biochemistry, Cell and Molecular Biology, Microbial Physiology, Enzymology, Hormone Biochemistry and Organic Chemistry at undergraduate level. For postgraduate courses, he taught Protein Structure and Function, Research Methodology and Research Topics.

Dr. Nor Aripin was appointed Associate Professor in 1993 and held the post of Head, Department of Biochemistry and Microbiology, Faculty of Science and Environmental Studies from 1995 till 1999. He then was appointed Deputy Director (Research Grant Unit), Research Management Centre from 2003 to 2007. Dr. Nor Aripin was appointed as a Professor in 2005, and from 2007 holds the post of Deputy Dean, Academic and Student Affairs, Faculty of Biotechnology and Biomolecular Sciences.

Dr. Nor Aripin Shamaan started research in Biochemical Toxicology in 1984 with a modest grant of RM6000 from Dow Pacific Chemical Company (M) Bhd into the distribution and penetration of Dowco herbicides in lalang and the cow grass. From then on, he continued his research into carcinogenesis and the biochemistry of pesticides in insects, plants, animals and microbes. Working closely with the Medical Faculty of Universiti Kebangsaan Malaysia, he carried out his research into

the detoxication enzymes in carcinogenesis. He was a consultant for Cerebos (M) Sdn. Bhd from 1996-1998, and a resource fellow for research quality assurance in Universitas Gadjah Mada, Jogjakarta, Indonesia in 2004-2005.

In extension and public services, Dr. Nor Aripin is active in the Malaysian Biochemical Society, holding the post of Secretary in 1986, and continuously a member of the Exco of the Society. From 2003 to 2005, he was the President of the Malaysian Society for Biochemistry and Molecular Biology. He is a life member of the Society.

Dr. Nor Aripin had also represented UPM in many committees, notably the Cluster Working Group in the Industrial Task Force, Pharmaceutical Sector in MIDA (1995 to 1998), and the Perak State Education Planning and Development Committee (1996-1997).

He is a member of several editorial boards mainly the Journal of The Malaysian Society for Biochemistry and Molecular Biology, Pertanika Journal of Science and Technology, and reviewer for Asia Pacific Journal for Biotechnology and Molecular Biology, and the International Journal of Medicinal Mushroom. He is an external examiner for Universiti Kebangsaan Malaysia, Universiti Malaya and Universiti Malaysia Sabah.

Professor Nor Aripin is married to Wan Zurinah Wan Ngah and has five children; two boys and three girls. He is now a proud grandfather.

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