

THE PROBLEMS POSED BY XENOBIOTICS IN CHEMICAL MIXTURES AND THE ROLE OF MIXED FUNCTION OXIDASES.

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This review aims to present in simple terms the health and environmental problems posed by exposure to the steadily increasing presence of xenobiotic compounds.

Investigations and previous reviews, which dealt with the functions of cytochrome P450-Mixed Function Oxidases with particular reference to their actions on chemical mixtures, are evaluated in detail. Special attention is also directed to investigations which deal with the evaluation of the toxicity of chemical mixtures.

The ever increasing quantity and variety of chemical compounds to which the public is inevitably exposed, makes it obvious that for effective evaluation of health effects due to chemical mixtures the classical toxicological methods, which only focus on the toxicity of individual chemicals, are inappropriate. Chemical mixtures may produce additive, synergistic or also antagonistic effects due to the induction and action of mixed Function Oxidases. Hence it is essential to consider the overall exposure to chemicals in evaluating the effects of chemical mixtures on public health and on the ecology.

Introduction

Xenobiotics (chemicals which are foreign to life) are now ubiquitous. About 65,000 chemicals are considered to be in common use (Anon. 1990). The quantity of organic chemicals produced annually (about 1 billion tonnes) will have increased about a 1000-fold between 1930 and the year 2000 (Anon. 1988).

The toxicity of approximately 80% of these commonly used chemicals has never been evaluated (Anon. 1990), and the toxicity of chemical mixtures is virtually unknown.

The availability of the many products of the chemical industry has often made life far more convenient, particularly for western society, but there is a price to pay.

The following discussion illustrates some of the recognized problems in the assessment of toxicity, the mechanisms and consequences of chemical interactions, and the role of the cytochrome P450 Mixed Function Oxidases (CYP450-MFOs) in the detoxication or bio-activation of xenobiotics.

Assessment of toxicity

In the past, the health effects caused by individual chemicals were able to be identified by methods elucidated by classical toxicology. These techniques focussed on single chemical substances, usually tested on highly inbred laboratory animals. The ubiquitous presence of chemical mixtures has made the classical approaches in many instances inappropriate. The concepts and subsequent regulations, developed from classical toxicology applying to exposures to single chemicals, such as Acceptable Daily Intake (ADI), Minimal Risk Level (MRL), No Observable Adverse Effect Level (NOAEL) etc., provide unrealistic safety margins in the present day environment. Gradual recognition of the limits of current toxicology is well expressed in the following

statement - "However a major wrinkle in our entire testing program is that each agent is tested separately and judgements about their acceptability are made on that basis. Unfortunately humans are not exposed as experimental mice and rats were, to just one agent alone, but to an entire array of chemical agents, in fact a sea of generally low levels of chemical agents. The issue for human health, therefore, is not just the likelihood that a simple agent will cause adverse health effects, but also how our testing and predictive systems deal with the reality of exposures to the complex mixtures within the sea of environmental toxins. Calabrese 1991).

The problem then for the medical profession and the regulators of chemical usage, is to seriously consider the effects of chemical exposure.

At a recent toxicology conference the obvious was stated - "Today, it is widely recognized that considerations of adverse health effects caused by exposures to chemical mixtures must be an integral part of protecting public health" (Sexton et al. 1995). The growing appreciation of problems posed by exposure to chemical mixtures is underlined by the recent emergence of books and guidelines on that topic. (Calabrese, 1991; Anon. 1951; Vouk et al. 1987; Ashford & Miller, 1991; Heuser et al. 1993; Altenburger et al. 1993; Pollak, 1993; Yang, 1994; Rea, 1994; Anon. 1996). However, most members of the medical profession still do not appreciate, understand or recognize the impact that the ubiquitous presence of xenobiotics has on human health.

Mechanisms of chemical interactions

The interactions of chemicals in mixtures may result in additive, synergistic and also antagonistic effects, as well as direct chemical and biological interactions.

Additive effects usually occur when chemicals with similar structure or action are present in a mixture, each chemical adding to the combined effect. Significantly even concentrations which are well below the so-called NOAEL of each individual chemical can contribute to the combined effect, and the sum of the concentrations of the individual chemicals in the mixture may then exceed the toxic threshold levels.

Synergistic interactions of chemicals in mixtures are usually due to changes brought about by one or more components in the mixture, changing the metabolism of other components and thus significantly enhancing the overall toxicity of the mixture, producing a multiplier effect, rather than just producing additive toxicity.

An example of the synergistic effects of contaminants present in low concentrations has been demonstrated by the U.S.A. National Toxicology Program (Yang et al. 1989). In one survey, 25 inorganic and organic compounds were identified as components of the toxic chemical mix contaminating ground-water. This mixture served as a 'stock' solution. A 10% solution of this 'stock' was provided as drinking water for mice. After 3 months significant impairment of the immune response of the mice was demonstrated, by

measuring the suppression of bone-marrow stem-cell proliferation. In addition it was also shown that if the mice were exposed to a 10% 'stock' solution for 2 weeks prior to the administration of a single low dose of carbon tetrachloride (0.075 mL/Kg), severe liver damage resulted. However if the mice were not exposed to the 'stock' solution, the administration of the same single low dose of carbon tetrachloride produced no toxic effects (Yang et al. 1989).

In another investigation, using the "MICROTOX" assay to compare the toxicity of sediment extracts, it was found that many extracts with below or barely detectable levels of priority pollutants were highly toxic (Jacobs et al. 1993). The point made by the investigators being that on an individual extract basis, concentrations of priority pollutants as single entities, were poor indicators of the actual toxicity of the sediment extract (Jacobs et al. 1993).

Antagonistic effects were discovered over 40 years ago, when an experiment was set up to test if the incidence of carcinogenesis could be increased by exposing rats simultaneously to 2 carcinogens. It was found that feeding 3-methyl-4-dimethylamino-benzene, a hepatocarcinogen, together with 3-methylcholanthrene, (another carcinogen) abolished the hepatocarcinogenic action (Richardson et al. 1952) Subsequently it was discovered that 3-methylcholanthrene induced CYP450-MFO enzymes. These enzymes catalyzed the demethylation and also the azo link reduction of amino azo dyes to non-carcinogenic metabolites (Richardson et al. 1952).

Direct chemical interactions may occur, such as the formation of nitrosamines from nitrites and amines present in a mixture. Nitrosamines being an obvious threat to health.

Another example of an adverse interaction occurred when calcium sulphate filler used in the capsule form of the pharmaceutical "Dilantin" (the active principle being phenytoin), was replaced by lactate. The solubility of the drug phenytoin was increased, in turn leading to increased bio-availability and hence to toxicity (Tyrer et al. 1970).

Biological interactions at the cell membrane level may also give rise to enhanced cytotoxic effects due to increased permeability afforded to a hydrophilic (water-soluble) chemical by lipophilic (fat-soluble) components in a mixture. These lipophilic components can damage biological membranes and thus increase the uptake of a toxic hydrophilic agent (Witte et al. 1995).

Cytochrome P450-Mixed Function Oxidases (CYP450-MFOs) in detoxication and bioactivation

Within most cells several groups of CYP450-MFOs are present which are concerned with the detoxication of toxic chemicals. Liver cells usually have the greatest concentration of these enzymes, lower concentrations are found in most other tissues, with the notable exception of skeletal muscle and erythrocytes. CYP450-MFOs are usually localized on the membranes of the smooth endoplasmic reticulum, though mitochondria of some tissues also contain these enzymes.

At least nine CYP450-MFOs are known to occur in human liver (Guengerich & Liebler, 1985) and it has been estimated that between 30-200 genes may be involved in coding and regulating the synthesis of different CYP450-MFOs (Nebert & Gunzales, 1985)

The CYP450-MFOs act on a wide range of substrates. They hydroxylate many xenobiotic chemicals, which increases the water-solubility of the xenobiotics. As a result these modified compounds are then more readily excreted by the kidneys.

CYP450-MFOs constitute Phase I of an enzymatic detoxication system. The role of Phase I enzymes is to add oxygen to chemicals. The addition of oxygen is usually followed by the addition of a hydrogen atom, giving rise to hydroxide derivatives which are more water soluble. The hydroxylated compounds are then suitable substrates for Phase II detoxification. Phase II enzymes include glutathione transferase and similar enzymes. These convert the oxidized compounds into molecules which can be transported to the kidneys for excretion. Phase III is the detoxication process concerned with the excretion of the conjugated, hydroxylated compounds. This is an energy dependent process (Sheehan, 1994).

However, in many instances CYP450-MFOs cause the bio-activation of toxic precursors, by converting relatively non-toxic compounds into hydroxylated derivatives and epoxides, some of which are highly toxic. During this process Reactive Oxygen Species, such as superoxide, hydrogen peroxide, singlet oxygen, hypochlorous acid or ozone, are formed. All of these can lead to the production of Free Radicals, which have the potential to be toxic. Glutathione and other Phase II compounds, (which serve to form conjugates with the hydroxylated compounds, making these even more hydrophilic, so that they cannot re-diffuse back through cellular membranes) tend to become oxidized by reactive oxygen species. This has the net effect of making Phase II compounds unsuitable to act as conjugates.

As a result, the potentially toxic epoxide and peroxide derivatives of the xenobiotics remain within the cells or tissues, where they pose a health risk. (These aspects have been discussed recently in a review paper (Pollak, 1996).

Apart from their action on xenobiotic compounds, the CYP450-MFO enzymes also catalyse the hydroxylation of endogenous compounds such as Prostaglandins, biogenic amines and steroid hormones (Guengerich & Liebler, 1996).

CYP450-MFOs are inducible, this means that the presence of a particular xenobiotic will activate genes to initiate the syntheses of CYP450-MFOs. In most cases the chemical which caused the induction of CYP450-MFO also acts as substrate for that enzyme. However in some instances, a single chemical can increase the concentration and activity of several different CYP450-MFOs, while actually decreasing the level of another CYP450-MFO (Guengerich & Liebler, 1985). Also the specificity of any one CYP450-MFO can be broad, catalyzing the oxygenation of a number of different compounds. Obviously then, it is difficult to predict the outcome of an exposure to a mixture of chemicals, particularly as the increase in enzyme activity may vary between 2 - 100 times (Guengerich & Liebler, 1985). For example it was reported over 20 years ago, that a massive occupational exposure to DDT by factory workers in a chemical plant, caused a 57% increase in the excretion of cortisol from the body by means of the enhanced conversion of cortisol (Poland et al. 1970). This increased excretion of steroids was due to the enhanced conversion of cortisol to 6-hydroxycortisol - a consequence of increased CYP450-MFO activity, resulting from the induction of CYP450-MFO by DDT.

The serum and lipid concentration of DDT and its metabolites were 20-30 times greater in the study group than in a control population (Poland et al. 1970).

In another study it was demonstrated that the activity of benzo-a-pyrene hydroxylase in placenta of smokers was found to be about 60 times greater than in the placenta of nonsmokers. It was also demonstrated that due to smoking the half-lives of antipyrine, theophylline and caffeine were significantly decreased (Conney, 1982).

The interaction between different drugs and xenobiotics is well illustrated by an experiment which evaluated the effects that phenobarbital or benzo[*a*]pyrene had on the muscle relaxant drug zoxazolamine. The biological half-life of zoxazolamine in vivo is 9 hours in control rats, but if a rat had been treated previously with phenobarbital for 4 days, the half-life was 48 minutes. A single treatment with benzo[*a*]pyrene 24 hours before the test, reduced the half-life of zoxazolamine to 10 minutes (Conney & Burns, 1972).

In the same review, it was pointed out that treatment of rats with phenobarbital for several days resulted in significant increases in the production of polar metabolites of testosterone, androsterone-3,17-dione, progesterone, deoxycorticosterone, estrone and 17 β -estradiol. It was also shown that the administration of phenobarbital stimulated the urinary excretion of previously administered DDT in cows, sheep, goats, calves and pigs (Conney & Burns, 1972).

In other experiments, carried out more than 30 years ago, it was shown that a number of different organochlorine pesticides greatly stimulated the formation of polar steroid metabolites (Conney et al. 1966). In view of all that evidence, it seems that urine analyses of Olympic athletes may not necessarily indicate recent steroid administration, but alternatively may reflect exposure to xenobiotics which induce CYP450-MFOs. Hence exposure to xenobiotics and the subsequent induction of CYP450-MFOs may be the cause of increased amounts of polar steroid metabolites detected in urine samples.

In direct contrast, organophosphate pesticides strongly inhibit polar metabolite formation of steroids (Conney et al. 1966). It has been reported that the induction of a particular CYP450-MFO, namely CYP2E1, by one chemical, can result in the enhanced toxicity of other chemicals. (Raucy, 1995). In this report it was also noted that the administration of pyrazole, acetone or other aliphatic alcohols to rats caused a 15- to 30-fold potentiation of carbon tetrachloride hepatotoxicity when compared with untreated rats (Raucy, 1995).

The CYP450-MFOs may be divided into 3 main groups, according to the type of chemicals which facilitate enzyme induction.

1. Induction by aryl hydrocarbons, such as benzo-a-pyrene, dioxins, PCBS, DDT, etc.
2. Induction by drugs, such as barbiturates, (phenobarbital), anticoagulants (coumarin), digitonin, or quinine.

3. Induction by steroid hormones

The potential bio-activation of xenobiotics, resulting from CYP450-MFO induction, will be influenced by the composition of the chemical mixture in any exposure setting. Therefore it is obviously essential that accurate identification of the composition of any chemical mixture must occur if a reasonable evaluation of the toxicity or potential adverse health effects are to be made.

Toxicity and the individual

The genetic constitution of the organism, as well as some of the following components all have the potential to influence the outcome of exposures to mixtures of xenobiotics (Vesell, 1987):

Age	Cardio-vascular function	Occupational Exposure
Sex	Gastro-intestinal function	Disease
Pregnancy	Immunological function	Infection
Lactation	Liver function	Fever
Exercise	Renal function	Drugs
Dietary Factors	Albumin concentration	Immunization
Alcohol Intake	Stress	Sunlight
Smoking	Starvation	Seasonal variation
Barometric Pressure		

In evaluating the effects of a chemical mixture on a group of individuals, exposed to a mixture of chemicals in the work place, or any other environment, the life-style factors of each individual must also be considered, since such factors can significantly modify the overall effects of the chemical mixture to which the individuals were exposed.

Conclusions

It is absolutely fundamental to realize that we need to resist the temptation to believe that a single concentration of a particular chemical is equally toxic for all people. Quite the reverse is true; multiple host factors ... render certain people more, and others less, susceptible to toxicity from a particular concentration of an environmental chemical." (Vesell, 1987)

Many factors require examination when making risk assessments, or when evaluating exposures to chemical mixtures in a specific environment. The precautionary principle must be applied for all exposed individuals.

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