

The Changing Field of Toxicology

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The Changing Field of Toxicology

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The science of toxicology is currently undergoing a transformation in medicine in the 1990s. The new toxicology is significantly different in its tenets to the high-dose, single agent toxicology of the past fifty years. The latter, while startlingly successful in the laboratory and in preventing death and injury from acute occupational exposure, has proven inadequate in describing the type of pathology or illness patterns seen in the population exposed to complex mixtures of potential toxins over long periods.

Molecular epidemiology combines the fields of molecular biology, epidemiology, toxicology and clinical medicine, and uses diagnostic tools to assess early, reversible changes in organ function (as opposed to irreversible organ pathology). This approach has already yielded unexpected data about the potential toxicity of individual chemicals and complex mixtures of chemicals in the population, and holds the promise of revolutionising risk assessment and minimising damage from environmental contaminants.

An unexpected early finding is that long-term, low dose exposure to complex chemical mixtures may not lead to organ-specific damage, as high dose, single chemical exposure seems to do. Instead, the health problems which arise tend to depend on the genetics and biochemistry of the exposed individual. In particular, it depends on the capacity of their detoxification pathways and antioxidant protective systems (among others) to prevent such harm. Inadequate nutrition, stress, infection and genetic susceptibility are now implicated as secondary factors in increased health risks from toxic agents.

Toxicology (and the legal and public health policies which stem from it) has long focussed on cancer risk as the primary determinant of danger from chemical exposure. While clearly an important outcome, it represents only the minority of important adverse outcomes following toxic exposure. The permanent brain damage following chronic exposure to solvents, lead and pesticides, may prove to be our greatest short term price of chemical exposure. Diminished male fertility following in utero-exposure to xeno-estrogens (chemicals which mimic estrogen, but are derived externally and are not under biological regulation) may be of enormous significance in the final historical review of the damage caused by toxic agents in the twentieth century.

The uncomfortable fact is that toxins may act in totally different ways at low dose than they do at high dose. These varying actions at different dosages may be the ultimate determinants of cancer risk. High level, acute pesticide or solvent exposure usually leads to direct damage to cellular membranes, and may also be mutagenic. Low level chronic toxicity, however, may be mediated through totally different pathways, such as by altering cellular function through hormonal mimicking effects.

Thus, our revered "dose-response" curves, which provide apparent reassurance that current exposure levels are "safe", may be meaningless. The data are derived from high-dose acute exposure to single agents in laboratory conditions. Extrapolation is used to derive so-called "no-effect levels" from LD-50s (a dose, which by definition, kills half of the test animals), without any allowance for differences between high dose and low dose exposure mechanisms. Nor does it allow for essential differences in basic biochemistry and metabolism between humans and the test animals.

Most test animals, for example, produce ascorbic acid in response to hepatic toxins, and this may act as a protective antioxidant, minimising damage from oxidation of membranes throughout the body. Humans and other primates, however, lack the final enzyme for production of the protective ascorbate, and require increased dietary intake to provide similar protection. If this is not forthcoming (attaining the tissue levels achieved by test animals may require intakes of many thousands of milligrams in adult primates), then the extrapolation upon which we make our assumptions may have no basis whatsoever. There is also a growing feeling that background chemical contamination from the general environment, the workplace and the home may have risks to health unforeseen by animal testing, and that those risks would be primarily borne by the foetus, developing infants, the sick and the aged.

The developing foetus and children have rapidly dividing cells, a developing nervous system and poorly developed detoxification pathways. The sick and aged suffer intercurrent illness, reduced antioxidant and detoxification capacity and depressed immunology. To apply the exposure standards, which have been derived from animal studies and gross epidemiological surveys to these high risk groups has little basis in logic.

Exposure to chronic low dose environmental toxins has been shown to increase cancer risk from all causes in adults, and has been associated with Chronic Fatigue Syndrome, altered haematological parameters, altered reproductive function in males (but not females), neuropsychological changes and abnormal psychiatric inventories in adults, permanent central nervous system damage, and altered immunological function in adults. While male fertility is generally decreased by exposure, immunology may be up-regulated or down-regulated depending on the agent in question. Neurological damage is most frequently associated with solvents, lead, and chemicals which interfere with neuro-esterases (especially organophosphate pesticides).

There are stages in life where cell division is naturally high, and these appear to coincide with the times of highest risk from mutagenic agents (see article - Cancer - An act in two parts).

The most critical time, clearly, is during foetal development, followed by early childhood, and followed again by puberty.

The extra risk for the developing foetus is in the development of the reproductive cells, the ova and sertoli cells. There is now some evidence that exposure to xeno-estrogens during the first and second trimester may lead to a decrease in sertoli cell numbers, and that this may lead to relative male infertility following puberty. If this is correct, then the apparent world-wide decrease in male fertility may be related less to current chemical exposure than to unregulated exposure during foetal development over the past two generations.

If the decline in human fertility is real, and is as significant as has been recently suggested, the consequences may be infinitely more significant in evolutionary terms than increased cancer risk in susceptible individuals. Finding ourselves at a reproductive dead end over the next few hundred years may well be a way of solving an overpopulation problem, but would be the ultimate irony in evolutionary terms. While the pests which we attempted to wipe out continue to thrive, the chemicals we used may yet be our own downfall through utterly unexpected mechanisms.

We should be cautious, therefore, not to extrapolate toxicity assessment outside the dosage range and species in which the testing is done. Rats tested for low-level exposure to dioxins (in the parts per trillion range) did not show increased tumour tendencies seen in the high dose exposure group, but did show a greatly increased incidence of endometriosis in the females when they were sacrificed. The high dose group, however, developed tumours but did not have the same increased incidence of endometriosis.

Conclusion

Toxicology is becoming a far more complex specialty than it was five years ago, but that does not mean it is incomprehensible for primary care physicians. The most exciting prospects for clinicians in the coming decade will be the ability to assess patients with early reversible health changes due to such low level contamination, and the ability to determine the type and extent of damage done, as well as the likely contributing agents, in a given individual. This field, known technically as "molecular epidemiology", and more popularly as "functional medicine", uses new diagnostic tools to:

- identify specific biochemical and genetic risk factors in healthy individuals. These appear to predispose to increased susceptibility to toxic exposure in the future.
- identify early, reversible changes in specific organ function (neurological, immunological, reproductive, hepatic, etc) occurring as a result of toxic responses.

Metabolic or biochemical profiling, first described in 1980, promises to provide a metabolic 'snapshot' of an individual's biochemistry, and this snapshot can serve as a baseline against which the effect of future toxic exposures, possibly occupational in nature, may be assessed.

The clinician already has access to a number of diagnostic tools to assess gross occupational and environmental contamination, and these remain useful to identify chemical-specific and organ specific risk. This type of risk, however, is simply the "tip of the iceberg", usually obvious and simple to identify.

The useful emerging technologies will allow the clinician to become actively involved in risk assessment and early intervention in the more common forms of population exposure to chemicals. Some diagnostic technologies, such as evoked response testing and assessment of metabolic pathways, are already available for those with an interest in the area, and will become more widely understood and available before the turn of the century.

Clinical Points

In practical terms, what can a practitioner currently do to ensure accurate diagnosis and best outcomes for people who are toxically affected? Some of the guiding clinical points should include:

- think of environmental and occupational chemical exposure as a possible cause of or contributor to the current illness. Failure to consider the possibility is the main cause of failure to make a successful diagnosis;
- take a full occupational and environmental history (to assess potential exposure patterns);
- use a formal environmental and health surveys to quantify risk and/or disability;
- arrange environmental health assessments (work, home or school) to assess contributing factors to toxic load, and determine avoidance strategies;
- assess detoxification pathways by formal biochemical testing (challenge / response);
- perform evoked response testing (AERP) on people exposed to solvents, lead or pesticides, those suffering multiple chemical sensitivities, and those with neurological symptoms;
- protect against further tissue damage by withdrawing the person from the toxic environment, and using broad spectrum antioxidants from optimal diet, nutraceuticals, herbs and nutritional supplements, before detoxifying;
- use a high quality, low allergy gastro-intestinal support and detoxification program, such as UltraClear. This is useful for those suffering toxic exposure or a disturbance of detoxification metabolic pathways.

The simple APDE principles, namely

- Avoid
- Protect
- Detoxify
- Educate

are most useful in determining a program of detoxication for an individual. The order is important. No amount of protection can beat avoidance of the problem and prevention. Never, never attempt detoxification programs without nutritional support and antioxidants. The chemicals are as dangerous on the way out as they were on the way in. Sometimes more so! And always educate your patients both about causes and early intervention, so they do not simply fall back into the same hole on returning to home and work.

Toxicology is moving inexorably from a process of identifying hazardous substances and their effects on a population, into the area of complex exposure patterns and individual susceptibility to such exposure. The outcomes for those "canaries", people at the leading edge of the bell curve, will be the first to improve. Greater emphasis on minimising unnecessary chemical use and exposure will ultimately improve the health of all, worldwide.

Metabolic Protection

Two processes in particular are worth mentioning in the context of individual susceptibility to toxic chemical agents. The first is the detoxification pathways within the body, and the second is the antioxidant capacity of the individual.

Detoxification (or detoxication)

The process of detoxification of xenobiotics (foreign chemicals) is not a simple one in humans. Probably developed as a first line defence against naturally occurring toxins in food, the detoxification processes occur mainly in the liver, but are part of the biochemical defences of most tissues in the body.

In broad terms, detoxification is a two stage process. The first stage, called Phase I, is one of making mainly lipophilic toxic compounds water soluble, and is typically mediated through the mono-oxygenase and cytochrome p450 enzymes, among others. This process often creates potentially toxic intermediaries, or partial detoxification products with higher potential toxicity than the original toxin. Phase II of the process is one of binding and removal of this intermediary through a wide range of possible pathways.

Probably the most important and best known is glutathione conjugation, although other pathways such as glucuronidation and glycine conjugation are also important. Reduced glutathione, often abbreviated to GSH, is a simple tripeptide of cysteine, glutamic acid and glycine. The sulfa-containing cysteine is the usual binding site for the partial products, and cysteine may be rapidly lost from the tissue if toxic exposure is high. Paracetamol poisoning, for example, rapidly outstrips the functional detoxification capacity of the glutathione, and the subsequent loss of cysteine in the excreted complex can be life threatening. Once the glutathione reserves are exhausted, hepatic damage rapidly becomes irreversible, and death will ensue unless cysteine stores are rapidly replenished (via oral or intravenous N-acetyl L-cysteine [NAC]), or the liver is transplanted.

An interesting sidelight here is that NAC is not available over the counter in Australia, although paracetamol is! In addition the TGA allows for the sale of L-cysteine hydrochloride as an over-the-counter nutritional supplement, despite its higher hepatic toxicity and lower efficacy when compared to the "banned" NAC. There would be a good case to be made for including NAC in every first aid kit in Australia, given that there are more preventable deaths from accidental or intentional paracetamol overdosing each year in Australia than there are deaths from snake or spider bites.

Each individual has a unique pattern of detoxifying processes at any given time, and these pathways are generally inducible. This means that constant exposure to toxins will tend to induce up-regulation of appropriate detoxification pathways if the person's genetics and nutrition allow for such up-regulation. Intermittent exposure, however, may prove to be more dangerous, even though the total dose is lower in intermittent exposure. With single high dose exposure, tissue damage may occur by suddenly overwhelming the protective pathways without inducing the protective enzymes. Even if detox pathways are activated, the time period between exposures may be long enough to see these fall to "normal" or even below. If this is followed by a further toxic insult while levels are low, the cumulative damage of intermittent exposure may be much greater than in a person who has the same total dose, but with continual exposure. In addition, intermittent exposure in some individuals may lead to the development of multiple chemical sensitivities.

The worst case scenario in terms of potential damage from toxic chemicals often occurs when the Phase I of the process is normal or even raised (ie induced), but is unmatched by the Phase II conjugation. This leaves the person under considerable toxic stress, and the carcinogenic and neurotoxic effects of the chemicals may remain high for long periods. Reducing toxic load from all sources (including gastrointestinal bacterial toxins and natural food toxins) can help, and are most effective when combined with a specific protective program such as the UltraClear program to ensure adequate quality nutritional intake.

Many years ago, we learned the mistake in our inpatient hospital unit of assuming that these people could cope with "normal" loads. We admitted many people who had suffered adverse effects from chemical exposure in the past. All, on admission, had normal liver function tests (LFTs), though many had been told by naturopaths in the past that they suffered "liver problems" or "toxic livers". We had

dismissed this on the basis of the normal LFTs, and proceeded to fast these people in the low chemical environmental unit.

Just under half of all out admissions developed abnormal liver function tests within 72 hours of admission to the hospital (we were doing blood testing for other reasons - this was simply a chance finding). Many of them were so severely abnormal that we suspected viral hepatitis at first, but this proved negative. What was going on? Our textbooks stated that a normal person should be able to go well over two weeks of fasting before the dropping glutathione caused a decrease in liver protection, and abnormalities of the LFTs.

We decided to put new admissions on a non-allergenic nutritional supplement known as Vivonex TEN (total enteral nutrition), containing trace elements, antioxidants and elemental amino acids, to try to avoid the problems of fasting. The problems with abnormal LFTs disappeared overnight, teaching us a valuable lesson as to just how close to the edge these sick people were in terms of their detoxification capacity.

We renamed our original approach the "liver stress test", making a virtue of a vice, and taking a leaf out of the cardiologists book!

One problem, however, was that Vivonex tasted so bad (due to the taste of free amino acids) that many patients preferred to risk liver damage rather than take it! The cost was in the order of \$450 per week as well, limiting its long term usefulness. We tried oligopeptide (two to five amino acids per molecule) formulae and other protein drinks as well, with little success, often because of the allergenic nature of the base proteins (milk, egg, etc).

Since that time, however, rice-based protein & carbohydrate formulae have provided the best and most acceptable product for the needs of most patients requiring improved detoxification. The cost of products like Ultraclear and Ultraclear Maintain is a fraction of the cost of Vivonex, allowing the patient to remain on the formula for the months which may be necessary for detoxification. The hypo-allergenic components on the formula also cause few problems for all but the most severely toxic and allergic patients, and the product specifically supplements and supports many of the detoxification pathways which may be compromised.

It is now also possible to test for the integrity of many of the most important detoxification pathways directly. This is done by a functional liver test, challenging a person with a few common toxins (caffeine, paracetamol, benzoate) in low dose and collecting the urine and saliva to assess the effectiveness of detoxification. This can be done in the USA at laboratories like Great Smokies Diagnostic Laboratories, and in Australia, a number of labs have taken up the methods pioneered in the USA.

In addition, specific therapeutic agents like the herb silimarin (St Mary's Thistle) have strongly protective effects in cases of toxic overload, and probably work by a combination of their antioxidant and detoxification actions.

Antioxidants

Aerobic organisms live on something of a knife-edge in our oxygen-rich world. Humans (among others) require oxygen for energy to sustain virtually all of life's processes every second we are alive. Without oxygen, and the mitochondria which use oxygen to create energy, we would die within seconds.

Despite this, oxygen is arguably the single most damaging pollutant on earth today!

Pollutant?

Most of the biomass on the planet died out over a billion years ago when a strange group of poorly mannered organisms, probably the blue-green algae, began photosynthesis for their own energy. The by-product of this new form of energy production was toxic oxygen. Over the next few hundred million years, organisms either died off, found niches which were oxygen free, or found themselves adapting to the increasing oxygen concentration in the atmosphere. Multicellular organisms began to emerge, and not only adapted to the oxygen in the atmosphere, but quickly became dependent on that oxygen for their energy. These were our real ancestors, and humans are among a select group of survivors of that original pollution problem.

Living with oxygen, however, is far from simple. Approximately one quarter of the energy our cells derive from oxygen is "re-invested" into protection against the harmful effects of that same oxygen. This is the so-called "oxygen-dependent antioxidant system", and comprises an enormous variety of enzymes and other chemicals whose job is simply to accept and donate electrons in a decreasing energy spiral, ultimately neutralising the potential damaging effect of highly oxidised chemicals within the cell and tissues.

Within the cells, chemicals like superoxide dismutase, catalase, glutathione (again), various peroxidases and ubiquinones are produced, each with a specific job in the antioxidant chain. From the diet, humans require additional antioxidants such as ascorbate (Vit C), Vitamin E, flavones and carotenoids to provide increased protection. Recently, it has been shown that the larger molecules such as albumin and other proteins also have a major role in "quenching" oxidative damage, and both urea and bilirubin are potent in this regard as well. Gilbert's syndrome (idiopathic hyperbilirubinaemia) may well be a gross indicator of oxidative stress in some people.

With such an enormous effort going into antioxidant protection, what could possibly go wrong? The fact is that no organisms in evolution ever survived by wasting energy on unnecessary metabolic protection. Put differently, the evolutionary survivors were those that did just enough to survive and procreate, and did not waste energy on extravagances such as extra (unneeded) antioxidant protection. Although we dealt with the oxidative load with minimal metabolic exertion for a million years, that may not be enough to get us through the twenty-first century unharmed.

Increasing petrochemical pollutants, and partial combustion products in particular, increase pro-oxidant chemicals (such as ozone) in the atmosphere. Decreasing use of fresh, organic fruit and vegetables diminishes antioxidant protection from food. Progressively increasing exposure to oxidants in air, food and water must clearly push us all towards our limits of antioxidant protection. So why don't we get sick?

The answer is that we do, primarily with degenerative diseases such as cancer, heart disease, arthritis, alzheimers disease, parkinsons disease, age related macular degeneration, immune defects and neurological damage. And it is in the potential prevention of these illnesses that antioxidants have their greatest potential.

The issue of getting the right balance of antioxidants is not a simple one, however, and we are now beginning to see the development of tests which can show who is low in both overall and specific antioxidant capacity, and who suffers increased oxidative damage.

These should allow physicians to tailor treatment (diet or supplements) to the precise needs of the individual, rather than take the shotgun approach to broad spectrum supplementation, or even worse, of specific inappropriate supplementation. The adverse effects of beta-carotene in lung cancer in Finnish smokers and pre-cancerous polyps in Australians could, and should, have been predicted on the basis of providing a growth stimulant (however good its antioxidant capacity, beta carotene is known for its ability to turn on cell division and proliferation) to people with damaged DNA.

In the meantime, a safe antioxidant regimen, supported by the medical literature, would include Vitamin E (500 to 750 IU per day), co-enzyme Q-10 (50 to 100 mg per day), ascorbate (250 to 1,000 mg per day), and selenium (preferably as selenomethionine or selenocysteine, 100 to 400µg per day). These doses are for adults, and should be reduced proportional to body weight for children.

The soluble B vitamins, other fat soluble vitamins (A, D, and K), carotenoids, flavones and flavonoids, trace elements and other antioxidants should usually be administered by a high quality diet, with adequate protein and essential fatty acid intake, if possible. This diet should be especially high in fresh, pesticide-free fruit and vegetables in season.

Where an high quality diet is impossible to achieve, where potentially toxic exposure is inevitable, or where the person is already ill, broad spectrum nutritional supplementation may be beneficial.

Cancer - an act in two parts?

According to Bruce Ames (developer of the Ames test for assessing carcinogenic potential of single chemicals), two separate processes appear to be necessary for the development of cancer. The first, mutagenesis (the ability to cause mutation of the DNA) is well known, and indeed has been the entire focus of many tests, including the Ames test. The second, mitogenesis (the ability to stimulate or cause the cell to divide) is less well appreciated, but is clearly a sine qua non of a proliferative process such as cancer.

The mutagenic potential of a single chemical has enormous variation from person to person, depending on cellular biochemical protection, whether the cell is dividing at the time of exposure, and the effectiveness of DNA repair. DNA damage alone, however, is more likely to cause the death of the cell than it is to cause cancer. The potentially cancerous cells must proliferate, and any agent which stimulates cell division in the period following DNA damage may be a critical factor in developing cancer.

Cell division is thus a risk factor in both mutagenic action of a chemical and subsequent uncontrolled proliferation. It is remarkable that this simple biological process has not been the focus of greater scrutiny over the years of cancer research.

Is mitogenesis an important factor in cancer development? The tentative answer is that it may be more important than the mutagenic potential of a toxin. Chronic infections provide an excellent model when assessing cellular proliferation in response to a noxious (but not necessarily mutagenic) agent.

Helicobacter pylori is known to cause chronic gastric ulcers, and this is a major risk factor for subsequent development of stomach cancer. *H. Pylori*, however, does not appear to be mutagenic in itself. How does it cause cancer? The uncomfortable answer appears to be that there are sufficient mutagens in the average western diet to cause the pre-cancerous DNA changes (nitrosamines, pesticides, etc), but these are unlikely to progress without some mitotic stimulus in adults. The chronic inflammation from the *H. Pylori* infection provides the mitotic stimulus, and in some people, gastric cancer is the outcome.

Other infections seem to act in concert with environmental toxins to produce different cancers. Among the best known is the increased carcinogenic potential of the aflatoxins in individuals with chronic hepatitis. It is almost certain that we will discover more and more infective agents, endemic to certain populations, as a predisposing factor for other cancers in the future.

The increased natural mitogenic activity in the foetus and young children makes them especially susceptible to environmental toxins. There is a growing feeling that teratogens and carcinogens may be part of a spectrum of potential outcomes of exposure in humans, rather than essentially separate processes. In other words, the critical difference between a teratogen and a carcinogen may be that of the timing of exposure.

The real risk of pesticides and other xenobiotics is that they appear to have an unfortunate combinations of effects which may dramatically increase their carcinogenic potential. This may be the reason for unexpectedly high lymphomas, leukaemias and tumours generally in agricultural workers and petrochemical workers.

At high doses, often due to accidental exposure, the chemicals act primarily as mutagens and can damage cell membranes and antioxidant protective systems as well as DNA. Left at this stage, many cells may die, but few long term consequences would be expected.

As the levels of pesticide drop, however, the chemicals begin to cause cell division through hormonal-mimicking low-dose effects. If the original damage is in hormone dependent tissue including the prostate, testicles, breast, cervix and uterus, cancer becomes the likely outcome.

Multiple chemical sensitivities

People who develop a controversial condition known as multiple chemical sensitivities (MCS), often do so following a single moderate dose exposure to volatile chemicals rather than following continual exposure at a similar dose.

While this condition is poorly understood, it is easily identified by the primary symptom of "pathosmia". This is a combination of a heightened sense of smell generally (hyperosmia) along with a sensation of disgust or aversion to certain aromas, at a level which the majority of the population would find innocuous. Often, this is accompanied by a constellation of symptoms otherwise indistinguishable from chronic fatigue syndrome (CFS), and some authors have noted the apparent link between the two conditions. One remarkably consistent noteworthy factor is the sudden and marked decrease in tolerance for alcohol. Sufferers can go from feeling fine with two to four drinks per day, down to severe adverse effects from a single drink. Few chemically sensitive people retain normal alcohol tolerance.

The controversy over multiple chemical sensitivities is thankfully diminishing as specific objective tests such as Auditory Evoked Response Testing and SPECT scans demonstrate clear and significant differences between MCS sufferers and the normal population.

The mechanisms are yet to be well understood (although direct neurotoxicity, especially in the limbic system, through olfactory pathways seems an attractive hypothesis with some experimental evidence at present), but the degree of disability and suffering for sufferers can be remarkable.

There is currently no consistently effective treatment for multiple chemical sensitivities. The practitioner can remove the sufferer from the main sites of exposure, establish tissue protection with broad spectrum antioxidants, and focus on other reversible complaints such as allergy and fatigue.

Drug treatment is rarely of benefit in multiple chemical sensitivities, and if medications are to be used, they should be commenced at a very low dose (generally 10% to 20% of the usual adult dose) to avoid the common adverse and paradoxical reactions to medications.

There is a suggestion that the neurological changes of multiple chemical sensitivities may be permanent in nature, and there are no data to refute this at present. AERP testing very frequently shows changes typical of high dose neurotoxic effects, even though the dose received by the person is clearly below the "generally accepted" toxic levels of exposure.

Clinically, multiple chemical sensitivities is similar to chronic fatigue syndrome in symptoms and prognosis. If anything, it is more frustrating for the practitioner to treat than is chronic fatigue syndrome, partly because of the inability to use therapeutic doses of treatments which one may wish to use, and partly because of the capricious nature of exacerbations and remissions, so closely tied to low level volatile chemical exposure.