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# Systemic Responses to Exposure to Chemicals and Their Responses as Indicators

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## 1 INTRODUCTION

Along with physico-chemical properties and the potential to elicit biological activity, the three routes of exposure (inhalation, contact, and ingestion) and the corresponding three portals of entry (the respiratory system, the skin, and the digestive system) determine to a large extent the fate and disposition of xenobiotics in the body (Hayes, 1984). Whatever the port of entry or the main target organ for attack, toxic chemical exposures induce an overall systemic response that is the integrated outcome of multi-functional involvement (Cralley and Cralley, 1985). Qualitative and quantitative evaluation of systemic effects also lead to opportunities to explore biochemical mechanisms of toxicity to obtain a greater understanding of biotransformation. Evaluation of systemic effects is necessary to assess the role of defence mechanisms, if any, employed by an organism against external toxic insult. These factors could prove of assistance in the assessment of risks from chemicals, provided the methods used for evaluating systemic effects are precise and adequately validated under different sets of exposure conditions (Klassen *et al.*, 1986).

The target organ affected is of paramount importance in understanding mechanisms of toxicity. Useful indications that a specific organ is the target of a toxic episode can be obtained from exploratory clinical examination. The effects must necessarily be dose-related to qualify as biological indicators of exposure.

Critical local concentrations have to be attained to elicit adverse responses at cellular, tissue, or functional levels (Hodgson and Guthrie, 1980). Thus, three WHO reports embody the principles to evaluate toxicity, provide guidelines for epidemiological studies on the health effects of environmental chemicals, and describe the special situation of prenatal exposures; they may be considered overviews of the methodological problems with linking exposure to effects observed in humans and in experimental animals (WHO, 1978, 1983, 1984). Methods to assess the effects of chemicals on reproductive functions have also been reviewed in SCOPE 20, SGOMSEC 1 report (Vouk

and Sheehan, 1983). This paper updates the review of methods currently used for systemic responses, other than reproductive toxicity, which have a potential to be used as biological indicators of chemical exposures.

## 2 DEFINITIONS AND CRITERIA

Non-adverse effects constitute the absence of changes in morphology, growth, development, and lifespan; non-adverse effects produce no impairment of the organism's capacity to counter additional externally applied stress (NRC, 1975, 1978). Furthermore, adverse effects constitute those changes that, following intermittent or continuous exposure, involve functional impairment and the inability to deal with additional stress. Chronic adverse effects persist for a long time, even upon cessation of exposure and upon the susceptibility of the exposed individual to the deleterious impact of environmental stresses other than those induced by chemicals. Many small, apparently non-adverse effects may collectively change into clinical disease, although there are no means at present to predict the time course of such an event.

The criteria used hitherto to demarcate adverse and non-adverse effects are based on quantitation of the impairment of vital physiological activities such as respiration, function of the heart, blood circulation, nerve transmission, reproduction, movement, digestion, excretion, and immune competence.

It is relevant to ask: could adverse effects be masked by non-adverse effects? There may be no gross change in pathology, but one can notice a change in organ function. In fact, Rudolf Peter's concept of "biochemical lesion as opposed to pathological lesion" underscores the possibility of non-adverse effects masking adverse effects (Glaister, 1986). The potential of using immunotoxicity tests (Descotes, 1986) to predict adverse effects induced by environmental chemicals as sequelae of non-adverse remains to be investigated.

In keeping with the above line of thought, pathophysiology of inflammation, necrosis, hyperplasia, and dysfunction has been emphasised in toxicity studies. Elucidation of mechanisms behind the subtle departures from normal functions has; however, received much less attention. Existing leads are relatively few to facilitate understanding how the cumulative impact of small variations results eventually in illness. Criteria derived from biochemical or metabolic alterations deserve the same degree of emphasis as has been assigned hitherto to overt pathological manifestations. To facilitate this, methods are needed to quantify the changes in turnover rates of enzymes, patterns of isoenzymes, and chemodynamics of substrates and their metabolites. A way is needed to predict the relative susceptibility of an



organism to the toxic effects of metabolic end-products and parent compound (Polson *et al.*, 1983).

### 3 NEED FOR EPIDEMIOLOGICAL METHODS FOR SYSTEMIC TOXIC EFFECTS

A good deal of recent research has sharpened our skills in making meaningful distinctions between adverse and non-adverse effects. Animal models have generated information on reversible versus irreversible changes, subtle departures from normal physiology and morphology, adaptive ability to stress, and diverse homeostatic control mechanisms (Calabrese, 1983). The toxicity of chemicals is widely variable even within a small size population (Gad and Weil, 1986). In this context, one has hardly to over-emphasise the need for methods to design, execute, and interpret epidemiological studies at the same time ensuring an internal mechanism to generate simultaneously quantitative exposure information. Adverse drug reactions have led to the development in recent years of a new branch of clinical epidemiology called pharmaco-epidemiology (Strom, 1987). The integration of methods is needed to correlate cause and effect, as developed by traditional infection-related epidemiology, to make meaningful observations on human populations exposed to chemicals as implied by the newly emerging discipline: environmental epidemiology.

A protocol based on a few critical diagnostic functional tests with adequate instrumentation support is expected to play a vital role in all future efforts to link exposure to delayed effects. Community-based studies using criteria such as standard mortality ratio or cancer incidence when designed to quantify adverse effects of exposure to chemical pollutants are plagued by confounding factors as illustrated by the investigations of human health effects on diesel exhaust (NRC, 1981; McClellan, 1987). Efforts are needed to refine the procedures for statistical evaluation and modeling in epidemiological studies related to chemical exposures.

Exposure to chemicals from the ambient, living, and working environments has assumed global dimensions with the rapid pace of industrialisation in the developing countries (Krishna Murti, 1981; 1983a). The increasing diffusion of potentially toxic chemicals in regions containing dense human populations in Latin America and Asia also represents the superimposition of chemically-induced stress on the burden already exerted by other environmental factors such as environmental sanitation, malnutrition, and coexistent infections and parasitic diseases. Do we have tools in our epidemiological kit to unravel the presumably complex outcome of the related interactions? The cluster of disease seen in such communities could be attributed to a web of causative factors rather than to a single identifiable agent. Risk assessment values developed in highly industrialised countries

and used for regulation of exposure to toxic chemicals may thus need innovative adaptation.

The series of chemical accidents of the last decade, ending with the Bhopal disaster, highlight the deficiencies in methodology for assessing health effects of single massive exposures to accidentally released chemicals, as opposed to methods in use for continuous exposure to sub-lethal levels of environmental pollutants.

#### 4 BIOLOGICAL MONITORING

Biological monitoring in the context of chemical toxicology attempts to establish relationships between internal exposure levels and the systemic effects elicited by chemicals. The complementarity of biological monitoring to environmental monitoring has been recognised (Naritz, 1979; Lauwerys, 1983; Vainio *et al.*, 1983). Absorption by all routes and from all media is taken into account while devising biological monitoring methods to provide a realistic base for arriving at estimates of total risk. Since biological monitoring is related to adverse effects, it is a more reliable indicator of risk than mere environmental monitoring of the levels of the causative chemical. Specimens routinely used for biological monitoring include blood and urine, breast milk, saliva, semen, and hair. Biopsy samples of adipose tissue have also been used occasionally. The potential of placenta as a tissue for biological monitoring deserves more serious consideration (Beaconsfield and Bridwood, 1982). Attempts have been made to establish relationships between placental levels of organo-chlorine pesticide residues and the incidence of voluntary abortion in a random sample analysis of samples collected from maternity wards (Saxena *et al.*, 1980, 1981a,b). Haemoglobin from RBC, albumin from serum, and peripheral lymphocytes have been useful in demonstrating covalent adduct formation. Refined techniques can make the quantitation of adducts a useful indicator of chronic toxicity, permitting monitoring of exposure to genetic agents (Bridges *et al.*, 1982; Vainio *et al.*, 1983).

#### 5 METHODS TO ASSESS SYSTEMIC TOXICITY OF CHEMICALS

Counting cases of mortality or morbidity in a population has been the method of choice for gathering information on the toxic effects of chemicals, and continues to provide the initial data in any accidental exposure to toxic chemicals. Records of mortality and morbidity maintained in national registries are useful in making statistical estimates, provided information on environmental levels of the implicated chemical are also available. Death



or cancer are referred to as “quantal” effects, i.e., they are either present or absent. A distinction has also been made between stochastic and non-stochastic effects elicited by an absorbed chemical depending upon whether they simply occur or manifest themselves adversely.

Chemical pneumonitis following a single, but severe, exposure to a lung irritant is an example of an acute effect. Progressive interstitial pulmonary fibrosis following repeated exposure to fibrinogenic dusts such as silica is a chronic effect. There are many other well recognised examples. Acute effects can also result from long-term exposures, e.g., epileptic convulsions after long-term exposures to dieldrin, myocardial infarction from exposure to CS<sub>2</sub>, and abdominal colic following lead exposure. Similarly chronic effects can appear after a sharp episodal exposure to air pollutants, particularly asphyxiants. Also effects can be specific or non-specific, and clinical manifestations are the integration of multifactorial effects (WHO, 1983).

### 5.1 QUANTAL EFFECTS

Advantages and disadvantages of using mortality and morbidity statistics and cancer registries have been discussed extensively in the WHO document on Environmental Epidemiology (WHO, 1983). National and regional registries need to be set-up in developing countries where a potential exists increasingly for chemical exposures. Regional cancer registries established by the Indian Council of Medical Research and the registries maintained for the last three decades in the Cancer Institute in Madras have been used to detect trends of cancer incidence linked to environmental factors including chemicals. The Department of Environment of the Government of India has activated a project to set up a registry of chemical accidents with a broader data profile than the one used by the Inspectorate of Factory Safety under the Ministry of Labour and Social Welfare.

### 5.2 RESPIRATORY AND CARDIOVASCULAR EFFECTS

Standardised symptom-based questionnaires and functional tests have been the two approaches generally used to elicit information on respiratory and cardiovascular effects (WHO, 1983). The questionnaire, standardised and tested by the American Thoracic Society and the Division of Lung Diseases of the United States National Heart and Lung Institute in respiratory epidemiology (Ferris, 1978), can perhaps be gainfully updated, and used in other situations of exposure through the respiratory system. The symptom-based questionnaire has been profitably used to gather preliminary information on prevalence rates of respiratory diseases associated with exposure to particulate dust and fibrous particles in industries in the organised and unorganised sectors of industries in India (ITRC, 1978–80).

Chest radiography and measurement of heart size are used to monitor effects on the cardiovascular system (Rose *et al.*, 1982). Risk factors such as serum cholesterol, blood pressure, smoking habits, relative body weight and ECG abnormalities (Holland *et al.*, 1979; Lebowitz, 1981) can be used to monitor effects on the cardiac activity of persons exposed to chemicals. Immunological reactions associated with air pollutants can be followed by seeking the existence of hypersensitivity. Rapid progress in immunology in recent years has concurrently led to the refinement of techniques to explore B and T cell associated immune mechanisms and alveolar macrophage function (Adams and Hamilton, 1984; Klaus and Hawrowics, 1984; Habu and Okumura, 1984). These have been used extensively in identifying the nature of obstructive pulmonary diseases and also immunological effects of chemical insult to the pulmonary system (Kay and Goetzel, 1985).

### 5.3 EFFECTS ON NERVOUS SYSTEMS

Structural or functional variations in the components of the central nervous system, the motor and sensory portions of the peripheral nervous system, or functional or organic disorders of the autonomic nervous system can lead to recognisable disorders. A large number of chemicals are implicated (Creese, 1982; Dewar, 1984). Disorders of the autonomic nervous system can also appear as disturbances of the cardiovascular system. A single lesion localised in the central nervous system gives rise to effects too obvious to be missed. A list of the available neurological test procedures as reviewed by Friedlander and Hearne (1980) is given in Table 1.

Neurotoxic agents may affect perceptive hearing and auditory balance. Mobile and stationary facilities for direct recording of audiograms, tape, or

**Table 1.** Neurological examination in exposure to chemicals

Method	Examples
Electroencephalography EEG	Styrene and mixed solvents
Nerve conduction velocity	Styrene and mixed solvents
Sensory nerve conduction velocity	Styrene and mixed solvents
Slow nerve fibrous conduction velocity	Lead and trichloroethane
Electromyograph EMG	Trichloroethane 2-Hexanone Mixed solvents, lead
Electroneuromyography	Mixed solvents
Specific questionnaires	Chlorodecone, methyl mercury, trichloroethane and manganese



disc systems, and filing in a computerised storage system are increasingly used in epidemiological surveys of impairment of hearing (Health and Safety Executive, 1978). Ambient noise and vibration are the main physical factors implicated in hearing disorders, and have naturally received relatively more attention. Very few studies have been reported on hearing impairment associated with a combination of physical factors and chemical exposures. Obstructive chronic inflammatory lesions in the nasopharynx ending up as conductive disorders or non-conductive deafness are presumably related to chemical exposures. They are, however, still in the stage of laboratory investigations. Feasible methods for epidemiological use may be expected to emerge out of these studies.

Symptom-based questionnaires (USDHEW, 1973) are used currently to study eye disorders in the community, and do not distinguish between chemical exposures and physical factors as causative agents. Bias introduced by the observer during examination can negate the interpretation; protocol designs have been described to overcome this difficulty (Elofsson *et al.*, 1980). Workmen engaged in the spraying of insecticides in the control of vectors in the public health sector in Lucknow, India, were examined by ophthalmoscopic aids; macular damage was noticed in a significant proportion of sprayers (Misra *et al.*, 1982). Circular areas of perifocal depigmentation ranging from 1/6 to 1/3 disc diameter were the main features of the damage. Fluorescent angiography according to Rosen (1972) and focal areas of hyperfluorescence corresponding to the lesion were observed. The fluorescein angiographic pictures suggested pigment epithelium defect. Electroretinography (ERG) is potentially useful to unfold the susceptibility of retinal rods and cones to chronic toxic injury of the eyes (Liverani and Schaeppi, 1979).

With advances in our knowledge of neurotransmitter and related receptor mechanisms, it is now possible to explore effects on most steps connected with nerve transmission (Damstra and Bondy, 1982). Postsynaptic and presynaptic receptors are under the modulatory control of the neuroendocrine system. These are obvious targets of attack by environmental chemicals. Certain nerve terminals contain more than one neurotransmitter. These cotransmitters could also be the target of toxicity (Lundberg and Hokfelt, 1983). Chemicals could impair the blood/brain barrier and possibly retard or accelerate local metabolism while exerting their neurotoxic action (Garattini, 1983). Winder and Kitchen (1984) have reviewed the relative advantages and shortcomings of several neurochemical methods in assessing the neurotoxic effects of lead.

Immunoregulation of the central nervous system (CNS) is an area where new methodologies would be most welcome. Ligand activation of substance P receptor sites on astrocytes generates arachidonate-derived proinflammatory and immunoregulatory compounds in a dose-dependent manner within the CNS. These findings suggest the interrelationship between the nervous

system and the immune system (Hartung *et al.*, 1988). This link could be the target of attack by chemicals.

#### 5.4 EFFECTS ON BEHAVIOUR

Generalised behavioural response to noxious environmental factors appears as the syndrome of irritability, depression, and diminished interest in the surroundings. These neurotic or emotional effects are often accompanied by dysfunction of the psychosomal system. Although they could also appear as a direct effect of chemical exposures, one cannot ignore the possibility of their forming a symbolic cover to the underlying physical impairment. Behavioural effects can, therefore, be considered as the integrated outcome of functional damage to the nervous system.

Qualitative and quantitative measures of mental state and behaviour are elicited by psychological and psychiatric screening questionnaires and structured interviews. These are supplemented with a battery of verbal and learning tests to quantify psychological and psycho-physiological performance. The relative merits of these tests in surveillance programmes and their cost/benefit evaluation have been discussed (Tilson and Mitchell, 1984). Some of these tests are enumerated in Table 2. The functions to be assessed vary from sensory alterations to motor deficits and associative dysfunctions. Due to the absence of appropriate guidelines, it has been a difficult task to correlate neurobehavioural functional deficits with specific neuropathological or neurochemical changes (Mitchell and Tilson, 1982; Mitchell *et al.*, 1982).

#### 5.5 EFFECTS ON THE HAEMOPOIETIC SYSTEM

It is known that  $200 \times 10^7$  RBC,  $120 \times 10^9$  granulocytes,  $20 \times 10^9$  lymphocytes, and  $150 \times 10^9$  platelets are lost from the blood stream every day; new cells from extravascular stores or produced *de novo* in the bone marrow replace the same number by the dynamics of the processes of degradation and synthesis. A large number of chemicals affect the related processes causing diverse types of blood dyscrasias (Wintrobe, 1981). Related biochemical reactions are mediated in an extremely fragile micro-environment which facilitates induction (by release from stem cells), maintenance, and regulation of cell replication, differentiation, proliferation, and maturation (Kelemen *et al.*, 1979). Very little is known about the molecular mechanisms operating behind these vital processes. Maturation of human erythroid cells involves the loss of affinity of the reticulocyte membrane to cyclic AMP as well as loss of its susceptibility to stimulation by epinephrine (Babu *et al.*, 1975). Standardised tests for measuring levels of cyclic-AMP and cyclic-AMP binding protein are readily available.



**Table 2.** Classification of behavioural techniques

Type of test	Advantages	Disadvantages
Screening	Cost effective; does not require extensive training of animals or personnel; permits testing of a large number of animals	Acquires intensive labour input; tends to be subjective and often yields less than interval scale data; may be relatively insensitive to subtle effect
Secondary	Typically automated and objective usually provides graded data; amenable to repeated measure designs; relatively sensitive to subtle neurotoxic effect	May be costly to perform because of equipment and training of animals and personnel, may not be amenable to testing large numbers of animals

The wide spectrum of end-points of toxic effects on the haemopoietic system include changes in

- (1) number of cells per unit blood volume,
- (2) cellular composition per unit of blood volume,
- (3) characteristics of stem cells in the bone marrow or spleen,
- (4) iron metabolism in erythrocytes, and
- (5) the turnover of humoral factors.

The humoral control on the three different types of haemopoietic cellular systems is indicated by the marrow transit time,  $t_d$  in circulation and number (see Table 3).

Classical haemopoietic toxicants such as benzene, lead, or alkylating agents, have been examined for quantitative changes in the above three parameters. Cellular dynamics confer on the system the inherent ability to tolerate a good deal of toxic injury, provided the stem pool is not reduced beyond its power of resilience. Simulation models using the above parameters and concepts have been used to propose new concepts but have not been used extensively to predict the haemopoietic effects of chemicals (Pabst, 1984).

**Table 3.** Dynamics of human blood cells

	Marrow transit time	$t_d$	Number
Granulocytopoiesis	8-13 days	6-7 hr	$4.4 \times 10^3/\text{mm}^3$
Erythropoiesis	4-7 days	120 days	$5.1 \times 10^6/\text{mm}^3$
Thrombopoiesis	4-10 days	9.5 days	$260 \times 10^3/\text{mm}^3$

Blood counts, bone marrow evaluation (in a limited number of cases), and functional tests are still valid for mapping toxic effects. Blood gas binding capacity is likely to be affected by structural alterations in haemoglobin or the plasma membrane, and can be a useful functional test. Since the biophysics of the cell surface is intimately connected with gaseous diffusion and uptake, scanning electron microscopy of RBC may be a useful diagnostic tool. Scanning electron microscopy has indeed been used to map qualitatively the surface changes in neonatal RBC induced by binding to bilirubin or its photodecomposition products. Bilirubin interacts with the outer half of the plasma membrane bilayer before haemolysis is induced. The membraneous crenation noticed is dependent on the dose of bilirubin (Kaul *et al.*, 1981).

## 5.6 EFFECTS ON SKIN

As the primary barrier between man and his external environment, the skin is a vulnerable target for the attack of toxic chemicals, and is also the port of entry of many chemicals which may not attack skin *per se* but may reach other internal targets (Suskind, 1977; Griffiths and Wilkinson, 1985). Multiple defence against penetration, fluid loss from the body, solar radiation, or physical trauma requires a tough structure and capability of being quickly replaced. The stratum corneum, the normal barrier, has a characteristic composition which permits the regulation of skin temperature and humidity.

Pathological effects on skin caused by chemical injury include inflammation, primary irritation, allergy, photosensitisation, pigment changes, and changes of sweat dynamics and release of waste products.

Techniques are available currently for quantifying and predicting all the above types of effects (Marzulli and Maibach, 1977; Harber, 1981; Pushvel *et al.*, 1982). Bacterial or fungal infections could aggravate a local skin lesion, and give a totally different appearance of the effects. Skin infections could also elicit immunologically induced adverse effects in distant sites (Champion, 1986).

Skin biopsies obtained from dermatology wards or plastic surgery units of hospitals have been used to construct models for binding and efflux of toxic chemicals used as dyestuffs (Joshi *et al.*, 1981, 1982). The lead for these techniques was obtained from earlier studies on binding of bilirubin to skin segments and collagen (Kapoor *et al.*, 1973; Krishna Murti, 1982).

## 5.7 EFFECTS ON ABSORPTION, DIGESTION, AND ASSIMILATION

The gastrointestinal system is the target of xenobiotics voluntarily ingested as well as those involuntarily consumed as pollutants along with water and



food. Exfoliative changes are the commonest among the effects of irritant chemicals on the esophagus. These have been used extensively in surveying the incidence of buccal cancer due to tobacco chewing (Sanghvi, 1981). The integrity of the tract mucosa is affected profoundly by xenobiotics. Infections, hypersensitivity, and malnutrition superimpose conditions which exacerbate the effects of toxic stress, and vice versa. The fate of chemicals in the tract is also under the influence of the symbiotic relationship of the commensal flora of the gut digestion and assimilation (Williams, 1972; Caldwell and Jakoby, 1983). Gut motility and secretion are controlled by the autonomic nervous system, and neuroendocrines by relatively complex mechanisms. The tract has a unique membrane structure to mediate the extremely elegant electrochemical processes: viz., acid production and secretion, ion transport, absorption, recognition of foreign material, the display of systemic immunity and tolerance, or hypersensitivity to chemicals. The enzymatic machinery of the enterocytes is fully equipped to handle problems of digestion as well as synthesis (Hoensch, 1982), and is a likely candidate for the toxic attack of chemicals.

Toxic effects exerted by chemicals in the tract tend to be non-specific. Nonetheless, mucosal biopsy specimens from the esophagus, stomach, jejunum, sigmoid colon, and rectum have been used to examine gross morphological changes as well as to quantify villus height, crypt depth, and surface area. Cellular infiltration can be measured by the use of specific stains; monoclonal antibodies can be used to obtain measures of mitotic indices and immunological identification of lymphocyte subsets. Extensive information is available on the gastrointestinal effects of acrylonitrile, polychlorinated phenols, and asbestos (Szabo *et al.*, 1983; Meek and Grasso, 1983).

Gut microflora can bring about atypical biotransformation reactions. Microflora not only detoxify compounds toxic to the host but also generate toxic aglycones from relatively non-toxic glucosides (Williams, 1972). The flora exhibit wide ecological changes due to nutritional or climatic stresses. On the other hand, certain indigestible matter ingested along with common food can induce profound changes in the metabolic activity of gut flora (Rowland *et al.*, 1983). The fate of a given chemical can be traced by radioactive labeling using the loop technique giving satisfactory animal models for *in vivo* absorption studies (Sandhu *et al.*, 1981).

Tests to evaluate mucosal integrity include differential absorption of poorly metabolised carbohydrate probes of different molecular weight (differential role of excretion of lactose, rhamnose or mannitol). Assay of  $\alpha$ -antitrypsin in stools helps in screening enterocyte damage. Tests based on quantitation of (a) acid production in stomach, (b) pancreatic secretion, or (c) B<sub>12</sub> absorption can also be employed when more specific answers are required. Non-invasive techniques, using telemetry, are on the forefront for measuring gut motility and the electrical activity of the tract.

## 5.8 FUNCTIONAL EFFECTS ON LIVER

As the primary organ responsible for biotransformation and chemical homeostasis, the liver deserves special attention. More than 90 to 95 percent of the bulk of liver is made up of hepatocytes which naturally receive the maximum exposure to chemicals. The pathological effects of disturbed hepatic function following chemical insult include impaired hepatocellular function, biliary obstruction, and portal hypertension.

Direct injury to hepatocytes leads to selective or total loss of function, manifested as alterations in nitrogen balance and failure to remove or conjugate bilirubin. It also results in build up of porphyrins and reduced synthesis of plasma proteins. Hepatocytes of the periportal area are recognised to be metabolically more active. However, hepatocytes of the centrilobular region contain high levels of cytochrome P-450, and respond readily to induction by drugs. The hepatocytes are also endowed with the capacity to create ligands non-specifically with many chemicals (Slater, 1978). Along with conventional tests for hepatic function including the calculation of  $t_{1/2}$  values (half-life values) for marker chemicals, whole body proton nuclear magnetic resonance scanners have been used to explore chemically-induced changes in hepatic function.

IgA secretory component, bile acids, albumin,  $\alpha$ -macroglobulin,  $\alpha$ -fetoprotein and  $\alpha$ -glycoprotein and hepatoglobin constitute the spectra of non-enzymatic indicators of hepatic function. It is likely that specific immunological tests for these markers will be available in the near future to evaluate the effects of environmental chemicals on hepatic function.

Partial hepatectomy has provided a valuable experimental tool for distribution and mechanism studies. In distribution profile studies with radio-tin  $^{113}\text{Sn}$ , significantly lower levels were observed in hepatectomised rats 72 hours after administration of a single dose of tin, suggesting either the inability of regenerating liver to cope with the challenge or accumulation by the kidneys. A compartmental shift of radio-tin from the cytosol to microsomal fraction was also observed (Dwivedi *et al.*, 1983a).

Non-enzymatic lipid peroxidation also decreases in hepatectomised rats, but returns to normal values as the rats recover; whereas, the total content of glutathione in liver shows exactly the opposite effect (Dwivedi *et al.*, 1984). A parallel also seems to exist between the increase in glucuronidation and deglucuronidation in regenerating liver (Dwivedi *et al.*, 1983b). Non-invasive techniques based on these observations could perhaps be developed for monitoring the impact of chemical stress on liver function.

Conjugation with glutathione and excretion as mercapturic acid derivatives are recognised as a major pathway in the elimination of toxic electrophilic compounds (Boyland and Chasseud, 1979; Habig *et al.*, 1974a,b). Enzymatic and non-enzymatic conjugation mechanisms are presumably involved in the detoxication of acrylamide. The conjugation activity showed a twofold



increase during growth from birth to adulthood. The relatively stable binding to the liver ligand may also be significant in the non-conjugate disposal or intracellular transport of electrophilic toxicants (Dixit *et al.*, 1980, 1981). Quantitation of related mercaptan derivatives in urine may be useful to assess effects of chemicals on liver function.

## 5.9 TESTS OF KIDNEY FUNCTIONS

Renal injury inflicted by chemicals is reflected functionally as proteinuria, glucosuria, and increased blood urea nitrogen (Piperno, 1981). The accumulation of organic anions such as *p*-aminohippuric acid and tetraethylammonium is correlated with histological changes in cortex (Miyajima *et al.*, 1983). It is also recognised now that a number of chemicals can be metabolically activated to cause nephrotoxicity (Rush *et al.*, 1984).

Acute renal failure is caused by nephrotoxic agents, e.g., mercury, chromium, arsenic, and ethylene glycol. Glomerular or tubular injury manifest as subchronic or chronic effects are excreted by hydrocarbon solvents including kerosene. Tubular injury with suppression of tubular reabsorption is also a common chronic effect associated with lead, mercury, cadmium, uranium, and bismuth. The method of choice for assessing tubular function in chemical exposures is the estimation of  $\beta$ -2-microglobulin and retinol binding protein (Bernard *et al.*, 1982). Haematuria has been routinely followed in workers and their families exposed to the manufacture and formulation of synthetic dyes in India.

## 5.10 EFFECTS ON THE ENDOCRINE SYSTEM

The endocrine system is associated with four important functions of the body: growth and maturation, regulation of physiological responses to external stimuli imposed by physical and chemical stress, regulation of reproductive processes, and maintenance of homeostasis.

The endocrine system operates with high precision, specificity, and efficiency. The system made up of the pituitary, thyroid, adrenals, endocrine pancreas, and the gonads is very sensitive to the toxic effects of chemicals. The interaction of chemicals with the endocrine system at the structural level could lead to a variety of metabolic and neoplastic changes (IARC, 1979). Some well documented examples are: effects of lithium used in the treatment of manic-depressive disorders (Bagchi *et al.*, 1982), the occurrence of thyroid dysfunction by polyhalogenated biphenyls (Barsano, 1981), and action of anti-thyroid agents (ethylenethiourea and thiourea) on thyroid function (Graham and Hansen, 1972; Graham *et al.*, 1973).

With techniques for producing species-specific antibodies, it is possible to assay circulating hormone levels by radioimmunoassay and enzyme linked immunoabsorbent assay (Boorsima, 1984). Weighing glands and routine

histology provide a good screening test for sodium bromide and organotin toxicity (Leeuwen *et al.*, 1983; Lobber *et al.*, 1983; Funabashi *et al.*, 1980).

A single functional test integrating all endocrine functions is at present beyond practical realisation. However, for specific reactions modulated by a given hormone, the following release tests have been recommended:

- (1) for anterior pituitary function—release of LTH or FSH;
- (2) secretion of TSH and prolactin into serum;
- (3) release of corticosterone; and
- (4) release of insulin.

Among the non-invasive techniques are uptake of parenterally administered  $^{125}\text{I}$  or  $^{135}\text{I}$  by thyroid and body scanning and urinary release of 17-hydroxycorticosteroids. A critique of these methods can be attempted only after synthesis of all available information. Measurement of FSH or LTH release is useful in assessing pituitary function. Since these gonadotrophins are released in a non-continuous, pulsatile fashion, interpretation of plasma levels from a single time period may provide misleading results.

#### 5.11 EFFECTS ON MUSCULOSKELETAL SYSTEM AND GROWTH

Morbidity statistics have been the basis for assessing effects of environmental stresses on the musculoskeletal system. Chemical exposures of significance to growth and the musculoskeletal system include fluoride which leads to skeletal fluorosis, lead and the blue line on teeth, siderosis from excessive iron intake leading to spinal osteoporosis, osteoarthropathy associated with arsenic exposure, and secondary osteomalacia resulting from renal damage due to cadmium (WHO, 1983). X-ray scanning is the only technique available currently to evaluate musculoskeletal effects induced by chemicals.

#### 5.12 EFFECTS ON THE IMMUNE SYSTEM

Laboratory studies on rodents have shown that the immune system is very sensitive to chemical injury. Biological effects elicited by xenobiotic induced immune damage may become detectable only after a long latency. The attack by chemicals could be on (1) macrophage activation, (2) B cell maturation, or (3) T cell maturation leading either to altered host resistance to infectious diseases or new forms of allergy and autoimmunity (Luster *et al.*, 1987). Polybrominated biphenyls (Bekesi *et al.*, 1978) and polychlorinated biphenyls (Chang *et al.*, 1982) cause immune suppression. The presently used routine tests in experimental work are changes in weight and in the histology of thymus, spleen, mesenteric, and popliteal lymph nodes and changes in peripheral lymphocytes and monocyte counts (Vos *et al.*, 1983). Immune function tests include cell-mediated immunity, humoral immunity, and non-specific resistance (Vos *et al.*, 1984). Organotins exert a direct



cytotoxic effect on thymic lymphocytes; whereas, TCDDs injure the thymic epithelial cells (Greenlee *et al.*, 1984; Nagarkatti *et al.*, 1984). One could also include the highly sensitive and specific immunoperoxidase techniques for histochemical identification of hormones (Lobber *et al.*, 1983) as part of routine histopathology of biopsy specimens.

Improvements in the preparation of sections and histochemical identification have been published (Hancock *et al.*, 1982; Casanova *et al.*, 1983; Gendelman *et al.*, 1983; Franklin, 1984). The techniques used for identifying inherited and acquired immune deficiencies are also potential tools for investigations in immunotoxicology.

## 6 PERSPECTIVES

It is evident that a number of systemic effects, manifested as structural or functional changes, have potential to be used as biological indicators to assess chemical exposures. Ready availability of trained personnel and access to sophisticated instruments are the constraints in their wider application. It may be necessary, therefore, to evolve standardised procedures and relatively simple protocols for health surveys. They should have their basis on well established clinical studies related to exposure to the chemical in question. Health effects of air pollutants on the citizens of Greater Bombay have been studied by simple but reliable methods, and may be adapted for similar studies. Exposure through water and food intake will constitute the main risk due to environmental chemicals of the general population in the third world countries. These countries carry out routine health and nutritional surveys. It would be advisable to include a few critical parameters in such surveys as indicators of chemical exposures. There is need to emphasise the urgency of obtaining reliable norms of the indicators in select or high risk populations in these countries.

Observations of animal populations and cohabiting human settlements have also been very useful in revealing routes and deposition of toxic dusts and particulate in lymph nodes (Dwivedi *et al.*, 1981). Although studies on plants may not be directly relevant to assess effects of chemicals on human health, alterations in the viability and germination performance of seeds under different conditions of exposure to chemicals and pesticides display a very interesting profile of changes. These are of theoretical relevance to differentiation and development (Dwivedi *et al.*, 1981; Beg *et al.*, 1982).

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