CHAPTER II

BACKGROUND AND LITERATURE REVIEW

2.1 Organophosphate Pesticide

2.1.1 General Structure of Organophosphate

Organophosphate (OP) is one class of insecticides that have become the most widely used available nowadays. Organophosphate pesticides are used in agriculture to control pest, used in home, in garden, or in veterinary practices.

Organophosphates are classified in group of contemporary or non-persistent pesticides. By nature, OP pesticides do not persist in the environment; most decompose within several weeks with exposure to sunlight and water. In addition, these pesticides tend not to be bioaccumulated, therefore they are typically metabolized and excreted from the body in few days. The non-persistent pesticides are structurally diverse and have varied mechanisms of action (Barr and Needham, 2002).

OP pesticides are comprised of a phosphate (thio- or dithio) moiety and an organic moiety. In most cases, the phosphate moiety is *O*,*O*-dialkyl substituted. These pesticides are potent cholinesterase inhibitors. They can reversibly or irreversibly bind covalently with the serine residue in the active site of acetyl cholinesterase and prevent its natural function in catabolism of neurotransmitters. This action is not unique to insects, but can produce the same effects in wildlife and humans.

In a majority of OP pesticides, the alkyl groups (R_1, R_2) are represented by either methyl or ethyl groups, whereas, the chemical structure of X moiety defines the "leaving group", the majority of the structural difference between individual OP

pesticides (Figure 2.1). OP pesticides are often used the phosphate moiety in the 'thio' form (P=S) where metabolic oxidative desulphuration is necessary to produce an OP with anticholinesterase activity, the "oxon" form (P=O).

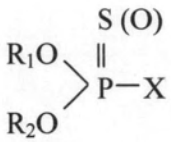


Figure 2.1 General chemical structure of organophosphate pesticides

2.1.2 Urinary Organophosphate Metabolites

Once human exposure occurs, OP pesticides are usually metabolized to the more reactive oxon form which may bind to cholinesterase or be hydrolyzed to a dialkylphosphate and a hydroxylated organic moiety specific to the pesticide. As a result of binding to cholinesterase, the organic portion of the molecule is released. The cholinesterase-bound phosphate group may be aged by the loss of the *O,O*-dialkyl groups, or may be hydrolyzed to regenerate the active enzyme. These metabolites and hydrolysis products are excreted in the urine within 24-48 hours of absorption.

Six dialkylphosphate (DAP) metabolites are the most commonly measured metabolite of OP pesticides, include dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), diethylthiophosphate (DETP), dimethyldithiophosphate (DMDTP), and diethyldithiophosphate (DEDTP) as present in Figure 2.2. Each of the six urinary dialkylphosphate metabolites can be produced from the metabolism of more than one OP pesticide. In addition to reflect parent pesticide exposure, the level of the metabolites in person's urine may reflect exposure to the metabolite itself, if it was present in the person's environment (CDC, 2003).

Urinary DAP metabolites are important markers of exposure to organophosphate pesticides in biological monitoring, particularly for exposure assessment in a general population, including children. (Weaver *et al.*, 1998; Heudorf and Angerer, 2001; and Wessels *et al.*, 2003)

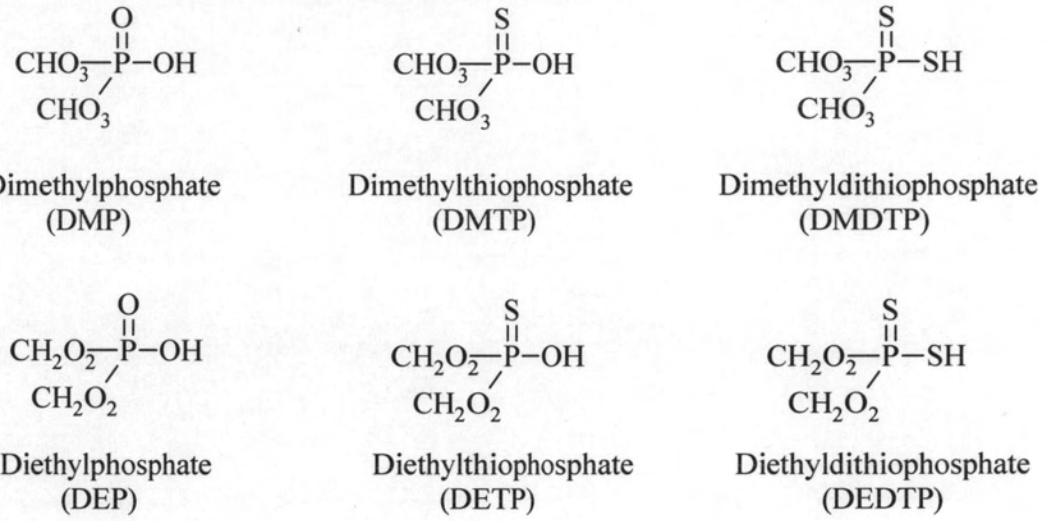


Figure 2.2 Chemical structure of the six dialkylphosphate metabolites

2.1.3 Toxicity and Health Effect of Pesticide

Acute Toxicity and Acute Effects

The acute toxicity of a pesticide refers to the ability of the chemical to cause injury to a person or animal from a single exposure, generally of short duration. Acute toxicity is determined by at least three methods: a) dermal toxicity is determined by exposing the skin to the chemical; b) inhalation toxicity is determined by permitting test animals to breathe vapors of the chemical; and c) oral toxicity is determined by feeding the chemical to test animals. The harmful effects that occur from a single exposure by any route of entry are termed acute effects.

Chronic Toxicity and Chronic Effects

Chronic toxicity is determined by subjecting test animals to long-term exposure to a pesticide. The harmful effects that occur from small doses repeated over a period of time, usually years, are termed chronic effects. Some of chronic effects found in test animals exposed to certain pesticides include birth defects, production of tumors (oncogenesis), genetic changes (mutagenesis); blood or nerve disorder; endocrine disruption; and reproductive effects.

Pesticide Poisoning

The most serious pesticide poisonings usually result from acute exposure to organophosphate and carbamate insecticides. Typical effects from pesticide poisoning are a result of either the irritant properties of a pesticide or an allegoric response by the victim. Dermatitis, or inflammation of the skin, generally is accepted as the most commonly reported typical effect associated with pesticide exposure. Some people tend to cough, wheeze, or sneeze when exposed to pesticide sprays. Both the active and inert ingredients in a pesticide may cause this reaction.

Symptoms of acute organophosphate poisoning develop during or after exposure, within minutes to hours. Exposure by inhalation results in the fastest appearance of toxic symptoms, followed by the gastrointestinal route and finally the dermal route. All signs and symptoms are cholinergic in nature and affect central nervous system receptors.

Cholinesterase Inhibitors

Organophosphate pesticides have adverse effects on insects and human by inhibiting acetylcholinesterase enzyme (AChE) at nerve endings result in a build up of acetylcholine (ACh), an important neurotransmitter which accumulates in synapse due to a jamming of information preventing messages from being passed properly between nerve cells. The result is a loss of available acetylcholinesterase enzyme so

that the effector organ becomes over stimulated by the excess acetylcholine. Acetylcholine accumulates in the synapse and there is a 'jamming' of information preventing messages from being passed properly between nerve cells.

Cholinesterase inhibitors prevent nerves from working correctly. This can affect the nerves in the brain, responsible for release of hormones or controlling hormones' actions. Since hormones are especially important in early stages of human development and in reproduction, such as endocrine disruption can be particularly damaging to human embryos or children (EJF, 2003).

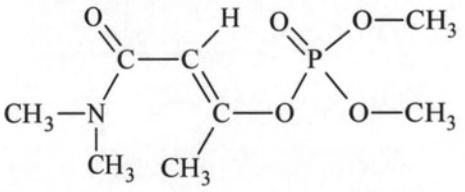
2.1.4 Organophosphate Pesticides of interest

The information for OP pesticides of interest described below based on various sources of pesticide information from Extension Toxicology Network (EXTOXNET online) and Pesticide Action Network (PAN online).

1) Dicrotophos

IUPAC Name: (*E*)-2-dimethylcarbamoyl-1-methylvinyl dimethyl phosphate or 3-dimethoxyphosphinoyloxy-*N*,*N*-dimethylisocrotonamide

Structure Formula:



Molecular Formula: $C_8H_{16}NO_5P$

Molecular weight: 237.21

Boiling Point: 400 °C

Melting Point: < 25 °C

Solubility in water: miscible

(Sources: Health Council of the Netherlands, 2003)

Chemical Class/Use: organophosphate/contact, systemic insecticide and acaricide

Physical Properties: a yellow-brown liquid with a mild ester order, corrosive to cast iron, mild steel, brass, and stainless steel, stable when stored in glass or polythene containers up to 40 °C.

Toxicity: highly acutely toxic, cholinesterase inhibitor, reproductive and developmental toxicity, neurotoxicity, no carcinogenic effects

Fate in Human and Animals:

Dicrotophos is metabolized in part to monocrotophos. Hydrolysis of the vinyl-phosphate bond of dicrotophos or its oxidative metabolites to produce dimethyl phosphate is the predominant detoxifying reaction in humans. In mammals, including rats, mice, dogs, rabbits, and goats, dicrotophos undergoes hydrolysis to dimethylphosphate. The residues of dicrotopos are excreted almost entirely within 24 hours, as indicated by a rapid decrease in not-hydrolysed metabolites in urine or milk.

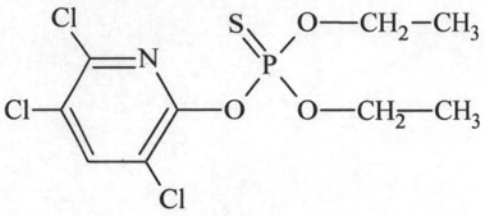
Environmental Fate:

The major routes of dissipation of dicrotophos in the environment are microbial degradation in soil and movement into surface and ground water. Dicrotophos degradation is not induced by exposure to light. Laboratory studies showed that dicrotophos was stable to photolysis in aqueous solutions (pH=7) and soil surface (sandy loam soil, pH=5.7). The haft-lives of dicrotophos in the aqueous and soil at pH 5,7, and 9 are 117, 72, and 28 days, respectively. Dicrotophos and its degradation products do not persist in the environment. In soil, dicrotophos is rapidly degraded under aerobic and anaerobic conditions. The haft-life of dicrotophos in sandy loam soil was 2.7 days and 7 days, respectively.

2) Chlorpyrifos

IUPAC Name: *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate

Structure Formula:



Molecular Structure: $C_9H_{11}Cl_3NO_3PS$

Molecular weight: 351

Boiling Point: 377 °C

Melting Point: 41.5 to 44 °C

Solubility in water: low; 2 ppm at 35 °C

Chemical Class/Use: pyridine organophosphate insecticide and organothiophosphate acaricide

Physical Properties: an amber to white crystalline solid with a mild sulfur (mercaptan) odor, violent decomposition at temperatures above 130 °C, corrosive to copper and brass, stable in neutral or acidic aqueous solutions

Toxicity: moderately toxic to humans, cholinesterase inhibitors, systemic intoxication, not reproductive effect, not carcinogenic

Fate in Human and Animals:

In humans, chlorpyrifos and its metabolites are eliminated relatively rapidly following a single dose. Its half-life in the blood after a single oral dose appears to be about one day. Following oral intake of chlorpyrifos by rats, 90 % was removed in the urine

Environmental Fate:

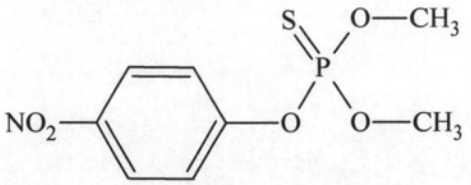
Chlorpyrifos adsorbs strongly to soil particles and it is not readily soluble in water. It is not mobile in sandy loam and loamy sand soils. Chlorpyrifos is less

persistent in the soils with a higher pH. Adsorbed chlorpyrifos is subject to degradation by UV light, chemical hydrolysis and by soil microbes. The haft life of chlorpyrifos in soil is usually between 60 and 120 days depending on the type of soil, climate and other conditions.

3) Methyl Parathion

IUPAC Name: *O,O*-dimethyl *O*-4-nitrophenyl phosphorothioate

Structure Formula:



Molecular Formula: $C_8H_{10}NO_5PS$

Molecular weight: 277

Boiling Point: 143 °C

Melting Point: 35 to 36 °C

Solubility in water: 55-60 mg/L at 20 °C

Chemical Class/Use: phenyl organophosphate insecticide

Physical Properties: a white crystalline solid with a characteristic odor of rotten eggs or garlic, react with strong oxidizers, decomposes rapidly above 100 °C and creating an explosion hazard

Toxicity: highly toxic by inhalation and ingestion, and moderately toxic by dermal adsorption, cholinesterase inhibitors, not carcinogen

Fate in Humans and Animals:

Methyl parathion is rapidly absorbed into the bloodstream through all normal routes of exposure. Following a single oral dose, the highest concentration of methyl parathion in body tissues occurred within 1 to 2 hours. Metabolism occurs in the liver, eventually to phenols which can be detected in the urine. Methyl parathion dose not

accumulate in the body. It is almost completely excreted through the urine within 24 hours.

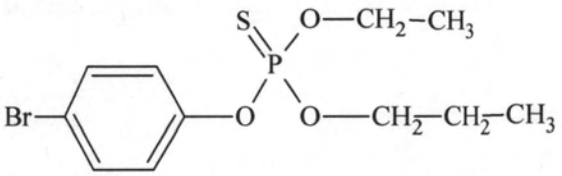
Environmental Fate:

Methyl parathion adsorbs to soil particles and degrades rapidly. When it is applied as an insecticide, methyl parathion breaks down within several months, primarily by photolysis and biodegradation. The rate of degradation increases with temperature and with exposure to sunlight. Its biodegradation haft-life in soil is 10 days to 2 months.

4) Profenofos

IUPAC Name: *O*-4-bromo-2-chlorophenyl *O*-ethyl *S*-propyl phosphorothioate

Structure Formula:



Molecular Formula: $C_{11}H_{15}BrClO_3PS$

Molecular weight: 373.65

Boiling Point: 100 °C (1.8 Pa)

Melting Point: (not available data)

Solubility in water: 28 mg/L

Chemical Class/Use: phenyl organophosphate insecticide

Physical Properties: pure yellow liquid with a boiling point 100 °C (1.8 Pa) and a density of 1.46 g/cm^3 at 20 °C , and amber colored oily liquid with boiling point of 110 °C (0.13 Pa), completely soluble in organic solvents at 25 °C (US EPA, 2000)

Toxicity: moderate order of acute toxicity following oral and dermal administration, cholinesterase inhibitors, systemic intoxication, not reproductive effect, not carcinogenic

2.2 Children's Health Risk

Children represent a sensitive sub-population or population at special risk in term of exposures to toxic compounds. Their exposures to environmental contaminants are expected to be different much higher than adults (Bearer, 1995; Goldman, 1995). Differences in exposure are due to differences in physiologic characteristics and also behavioral patterns, the way in which children interact with their environments, may have a profound effect on the magnitude of exposures to contaminants (Cohen Hubal *et al.*, 2000).

2.2.1 Children's Risk Characteristics

Children's physiologic characteristics influence exposure by affecting a child's rate of contact with exposure media or by altering the exposure-uptake relationship that governs internal dose resulting from an exposure. The physiologic characteristics influencing children's exposure (Bearer,1995) are given as follows:

1) Children have a much larger surface are relative to body weight than do adults. The surface area to body weight ratio for newborn infants is more than two times greater than that for adults. As a result of high body surface area, children are provided more area for dermal absorption.

2) Children need extra metabolic energy to fuel growth and development. It means that both oxygen and food requirements are greater per kilogram body weight for child resulting in greater inhalation and ingestion than for adult.

3) Children's bodies and brains are immature and still developing, they are more susceptible to certain cancers and reproductive problems, and they have a longer expected lifetime to develop illness after an exposure.

2.2.2 Children's Behaviors

Children's activities are considered to be important determinants of their actual dose of environmental contaminants (Cohen Hubal *et al.*, 2000a). Children often have greater contact with environmental contaminants because of their activities that involve contact with dirt and floor surfaces, and because of hand-to-mouth or object-to-mouth behaviors (pica behaviors).

Mouthing behavior generally includes all activities in which objects or hands are inserted into or touched by the mouth with the exception of eating or drinking (Groot *et al.*, 1998). A child's hands are the means for placing food in the mouth and are the immediate source of non-dietary exposure through hand-to-mouth and object-to mouth behaviors. Because the hand is used to act on the environment and probably has more contact with soil, dust, and water than any other part of the body, hands have been used as the equivalent of dermal surface in several studies (Zartarian *et al.*,1997; Gurunathan *et al.*,1998; Tulve *et al.*, 2002; and Black *et al.*, 2005).

Exposure to contaminants is also a function of the specific physical activities in which a child is engaged (e.g. playing games or watching television), the location of these activities (e.g. outdoor at home or in the living room), and the child's activity level while so engaged. Location where a child spends time determines the exposure media that may be contacted. Outdoor play activities of children often result in hand contact with the lawn, soil, or object on the ground (Weiss, Amler, and Amler., 2004).

2.2.3 Children's Developmental Stages

Children's exposures to environmental agents different associated with a child's age because of changing in location, levels of mobility, oxygen consumption, eating patterns, and behaviors. Thus, exposure assessments are required for children in each age group defined by the developmental stages, periods in a child's life. However, children age grouping varies from case to case when assessing exposure to environmental contaminants (US EPA, 2002).

There are in fact important exposure-related differences associated with a child's age (Needham and Sexton, 2000) as follow:

Infant (birth to 12 months)	contact with contaminated surfaces increase the potential dermal absorption
Young toddlers (1-2 years) Older toddlers (2-3 years) Preschool (3-5 years) School-aged (5-12 years)	hand-to-mouth behavior common for 1-3 years old, non-dietary ingestion from contaminated surface by 1-3 years, increasing time spend outside home, contact with contaminated soil surface, air, or water
Adolescence (12-18 years)	diverse diets and activity patterns, potential for occupational exposures

The other author (Bearer, 1995) different classified children's life stage as follows: newborn (0 to 2 months), infant/toddler (2 months to 2 years), preschool child (2 to 5 years), school-age child (5 to 12 years), and adolescence (12 to 18 years).

2.2.4 Children's Pesticide Exposure

Children's exposure to pesticides is a complex process that may occur from different pathways and routes. Figure 2.3 shows potential sources, pathways, and

routes of pesticide exposures that could occur. Sources include all pesticide uses that could result in children's exposure. Route of exposure (i.e., dermal, oral, inhalation) is defined as the portal of entry. Pathway is defined as the course that the pesticide takes from its source to the portal of entry. Exposure pathways will include those that occur indoor and outdoor at home, as well as non-residential settings such as schools or daycare centers (US EPA, 1999a).

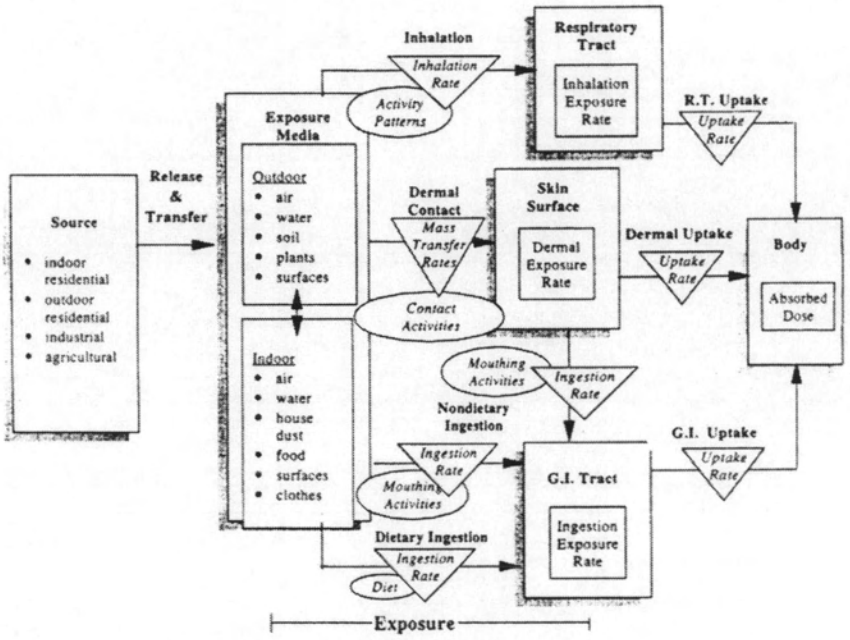


Figure 2.3 Children's exposure to pesticides
Source: US EPA (1999a)

Children can be at risk of dermal exposure to pesticides, both indoor and outdoor, because their behavior and lifestyles put them in frequent contact with many surfaces that may be contaminated with pesticide residues (Lewis,1994 and Fenske,1990). Dermal absorption of pesticides from housedust may also be a potential route of exposure for small children (Whitmore *et al.*, 1994; Simcox *et al.*, 1995). Moreover, because children spend more time in contact with soil and indoor surfaces than adult and have greater dose given the same exposure, these exposure pathways are particularly relevant to children (IPCS, 2000).

In addition, young children are particularly at risk from ingestion exposure because they exhibit frequent hand-to-mouth behavior, ingesting relatively large amounts of dust or soil (and any contaminants) in the process (Lewis,1994). Accidental ingestion of housedust for children may also be significant contributor to the exposure to toxic substance, depending on personal living conditions and frequency of contact with the media (IPCS, 2000).

2.3 Health Risk Assessment

Human health risk assessment is the likelihood or probability that a given chemical exposure or series of exposures will damage the health of exposed individuals. Health risk assessment is defined as the characterization of the potential adverse health effects of human exposures to environmental hazards (NRC, 1983). Human health risk assessment consists of four basic components (Figure 2.4) :

1) Hazard identification: describes the inherent toxicity of a risk agent

2) Dose-response assessment: describes the relationship between the dose and the magnitude, severity, or probability of a toxicological response.

3) Exposure assessment: estimates the level of current or anticipated human exposure.

4) Risk characterization: integrates the information from the first three components and estimates the potential risk as the probability or likelihood of adverse effects on a population.

When the health risk of a specific environmental hazard or situation has been characterized, regulatory decision must be made regarding which of the control actions should be taken. Risk management is formed as the core process to take the actions and make a decision in risk reduction and control (Reed, 2002 and Yassi et al, 2001).

Regarding the approach and the practices, the human health risk assessment process for pesticides is not different from the process for other hazard substances. However, because of the requirements for toxicology tests, pesticide risk assessment is unique in having a standard and extensive set of data for the risk evaluation (Reed, 2002).

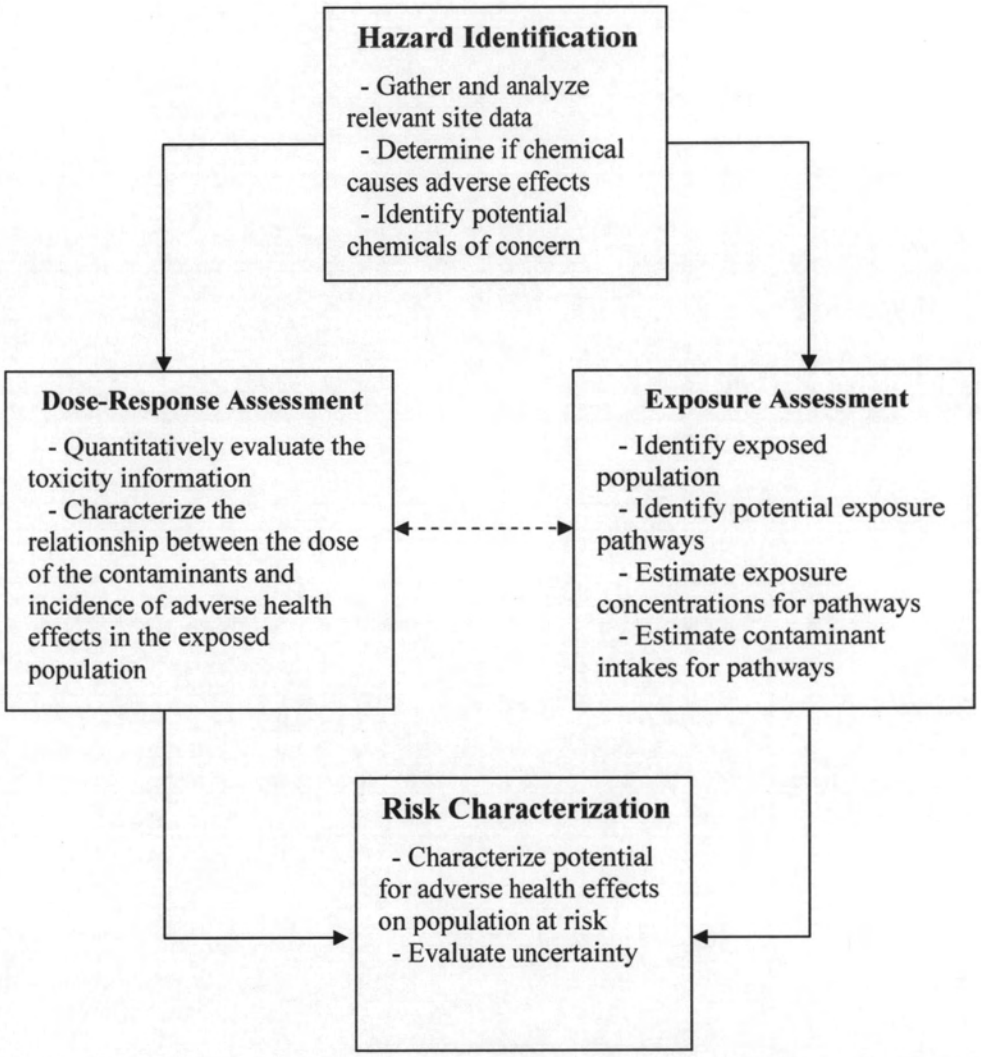


Figure 2.4 Component of the human health and chemical risk assessment process
Source: adapted from Asante-Duah (1993) and Reed (2002)

2.4 Exposure Assessment

An exposure assessment is conducted to estimate the magnitude of actual and/or potential human exposures to chemical constituents, the frequency and duration of these exposures, and the pathways by which humans are potentially exposed to chemical contaminants.

2.4.1 Definitions Regarding Exposure Assessment

Definitions for key events in exposure assessment (US EPA, 1992b) are summarized below.

Exposure : condition of a chemical contacting the outer boundary of a human with specific duration of time

Exposure pathway : a physical course taken by an agent as it moves from a source to a point of contact with a person

Exposure Route : the different ways the substance may enter the body, including dermal, ingestion, or inhalation

Exposure Concentration : a concentration of an agent in a carrier medium at the point of contact with the outer boundary of the human body

Most exposure assessments do not stop at exposure concentration, since that only information is not very useful unless it is converted to dose or risk (Sexton *et al.*, 1992). Assessments therefore usually estimate how much of an agent is expected to enter the body which can occur by either or both of two basic processes: intake and uptake.

Intake : Intake is associated with ingestion and inhalation. The agent, which is likely to be part of a carrier medium (e.g., air, water, food), enters the body by bulk transport

Uptake : Uptake is associated with the dermal route of exposure, as well as with ingestion and inhalation after intake has occurred. The agent, as with intake, is likely to be part of a carrier medium (e.g., water, soil, consumer product), but enters

the body by crossing an absorption barrier, such as the skin, respiratory tract, or gastrointestinal tract

Dose : Once the agent enters the body by either intake or uptake, it is described as a "dose." Several different types of dose are relevant to exposure estimation.

Potential (administered) dose : Potential (administered) dose is the amount of the agent that is actually ingested, inhaled, or applied to the skin. For the dermal route, however, it is important to keep in mind that potential (or administered) dose refers to the amount of agent that is applied to the surface of the skin.

Applied dose : Applied dose is the amount of the agent directly in contact with the body's absorption barriers, such as the skin, respiratory tract, and gastrointestinal tract, and therefore available for absorption.

Internal (absorbed) dose : The amount of the agent absorbed, and therefore available to undergo metabolism, transport, storage, or elimination, is referred to as the "internal" or "absorbed dose."

Delivered dose. The portion of the internal (absorbed) dose that reach a tissue of interest is called the "delivered dose."

Biologically effective (target) dose. Biologically effective (target) dose is the portion of the delivered dose that reaches the site or sites of toxic action.

2.4.2 Children's Exposure Assessment

Assessment of children's exposure necessarily involves three intrinsic factors including the influence of biological, physical, and social environments on the child's environmental health; variations in exposure-related attributes by developmental stage. In practical, both quantitative and qualitative approaches can be used to achieve the stated objectives and describe children's environmental exposure and related dose (Needham and Sexton, 2000).

Generally, exposure assessment can be conducted using either direct or indirect methods (NRC, 1991). It may be made in different ways, as illustrated in Figure 2.5.

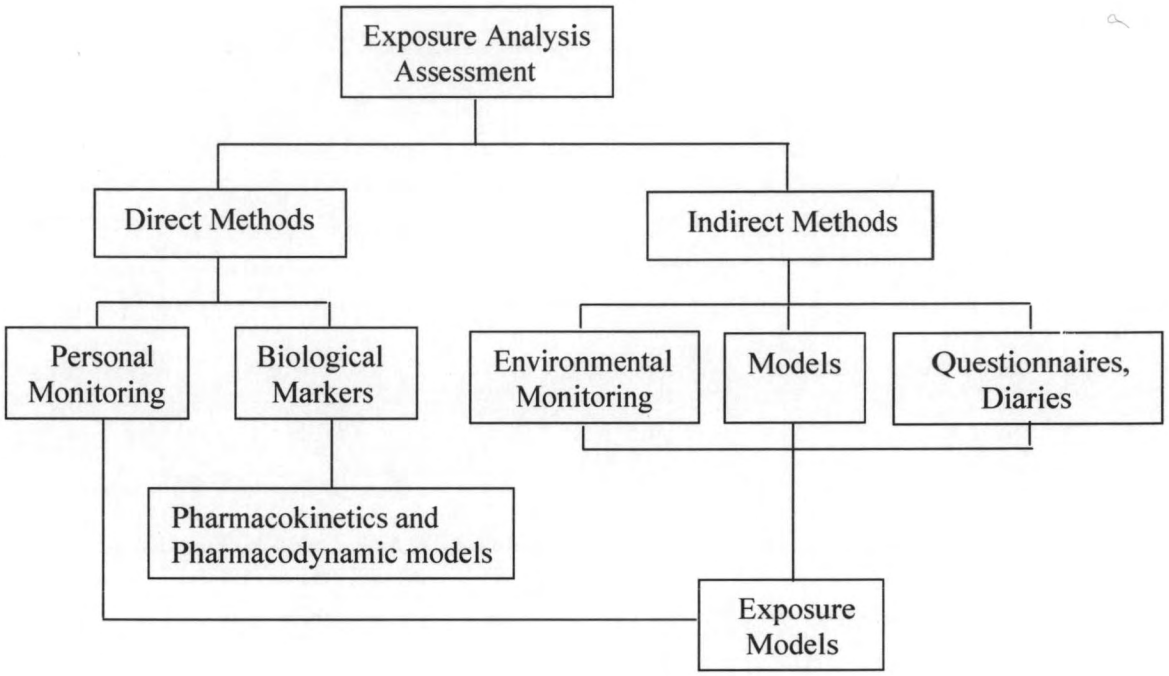


Figure 2.5 Possible approaches for exposures assessment
Source : NRC (1991)

1) Direct Measurements

Direct exposure assessment is to measure the contaminant concentrations at the point of contact between the child and the environmental medium. Personal monitoring and biological monitoring are considered direct approaches. *Personal monitoring* refers to collection of samples at the interface between the exposure medium and human receptor throughout a period of time. *Biological monitoring* usually measure dose, or more specially body burden at a point in time.

2) **Indirect measurement**

Indirect methods involve a combination of environmental sampling in relevant microenvironments through which children move during their normal routine (e.g. indoor at home), and data on the amount of time they spend in this microenvironment or engaging in exposure-related activities (e.g. play on the floor, hand-to-mouth activities) obtained from questionnaire (Needham and Sexton, 2000). *Environmental monitoring* provide information about the concentration of the chemicals to which children are potentially exposed and potential routes of exposure, whereas *questionnaire information* provides the data on the duration and frequency of exposure and timing of exposures (Needham *et al.*, 2005).

Indirect approach can be also involved models to quantify the relationship between pollutant exposure and important explanatory variables as well as for expanding existing exposure information to exposure estimation of a new population and future exposure scenarios (NRC, 1991). Therefore, environmental area monitoring as well as questionnaires, diaries, and mathematical models are considered indirect measure of children's exposure.

2.4.3 Exposure Measurements and Models

Direct measurements are the only way to establish unequivocally whether and to what extent individuals are exposed to specific environmental agents whereas it is neither affordable nor technically feasible to measure exposures for everyone in all populations of interest. Thus, models which are mathematical abstractions of physical reality, may obviate the need for such extensive monitoring programs by providing estimates of population exposures (and dose) that are based on a smaller number of representative measurements. The challenge is to develop appropriate and robust models that allow for extrapolation from relatively few measurements to estimates of exposures and doses for a much larger population (NRC, 1991).

For relatively small groups, measurements or estimates can be made for some or all of the individuals separately, and then combined as necessary to estimate the exposure (or dose) distribution. For larger groups, exposure models and statistics can sometimes be used to derive an estimate of the distribution of population exposures, depending on the quantity and quality of existing data (US EPA, 1992b).

2.5 Biological Monitoring

Biological monitoring is a tool for measuring pesticide exposure levels which entering the body. In cases where exposure fluctuates in time, and or the skin is a significant route of absorption, biological monitoring has proved for obtaining the absorbed dose information. In general, biological monitoring uses measurements in blood, urine, saliva, breast milk, or meconium as biological media by estimating the amount of pesticide as its metabolite or its reaction product that is absorbed into the body (Barr and Needham, 2002).

Current biological monitoring methods for organophosphate pesticides involve with two approaches. One is related to the inhibition of the enzyme activity of blood cholinesterases (plasma cholinesterase and red blood cell acetylcholinesterase). The second approach is to measure six dialkylphosphate metabolites which allow detection of absorbtion of the majority of organophosphates that have been recently in use (Manson, 2000).

2.5.1 Biological Marker

Biological indicators or biomarkers currently are available for biological monitoring pesticide exposure in human. As part of the risk assessment, biomarkers integrate routes, media, and pathways of exposure so that scientifically defensible risk characterization can be realized (Blancato et al, 1996). Biomarker measurement is

particularly important in children as their absorbed dose for a given external exposure level (Weaver et al, 1998).

According to the International Program of Chemical Safety (IPCS, 2000), biomarkers can be categorized into three classes as described following:

a) *Biomarker of exposure* : an exogenous substances or its metabolite that is measured in a compartment within an organism

b) *Biomarker of effect* : a measurable biochemical, physiological, behavioral or other alteration within an organism that can be recognized as a potential effect or disease.

c) *Biomarker of susceptibility* : an indicator of an inherent of an organism responding to a specific toxicant

Biomarker of exposure has been employed most frequently in industrial hygiene and occupational medicine before general use of biomarkers for exposure assessment of the general population (IPCS, 2000). It is an indicator of current present or past contact to environmental agents (Blancato et al, 1996).

Advantages of biomarkers for exposure assessment

Biomarker measurement has some usefulness for exposure assessing in children. Advance in the field of biomarkers may have important implications for the detection, prevention, and treatment of environmentally induced diseases in children (Bearer,1998). Biomarkers also integrate over all sources of exposure, which allows for efficient characterization of exposure to multiple sources and evaluation of historical exposures (NRC,1991). However, biomarkers can be the best way to measure recent exposures, especially those where dermal contact is the primary route of entry (IPCS, 2000).

2.5.2 Urinary Metabolite

Urine is one of matrix for biological monitoring. It's especially advantage when measured non-persistent pesticides or when biomonitoring in children is necessary or multiple samples are required. Non-persistent compounds are rapidly metabolized, and their metabolites are eliminated in urine (Barr et al, 2005). The presence of a contaminant or its metabolite in urine generally represents recent exposure. Another advantage of urine is the ease of sample collection, especially for studying in children, and the amount of sample available for analysis. In addition, non-persistent pesticides are usually found higher concentrations of their metabolites in urine than in blood due to their relatively rapid metabolism and excretion (Barr *et al.*, 1999; and Barr and Needham, 2002). The typical fate of non-persistent pesticides in human urine and blood is illustrated in Figure 2.6.

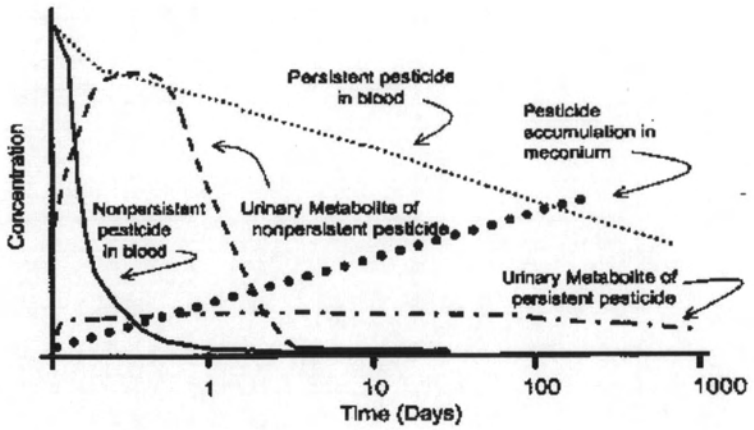


Figure 2.6 Typical fate of non-persistent pesticides in biologic media.
Source: Barr and Needham (2002)

Three types of urine samples are used for biological monitoring (IPCS, 2000) : *spot urine* samples are relatively easy to collect but there may be significant variability with respect to exposure prediction as a result of metabolism; *first morning void* samples have less variability since they are more concentrated than spot samples,

but require motivated subjects to collect the samples; *twenty-four hour urine* samples control much of the intra-individual variability but require highly motivated subjects.

First morning void urine has been generally selected to be a sample because it was found to be the best predictor of average daily metabolite concentration (Kissel *et al.*, 2005), and simply to collect from children. However, because of non-regulated body fluid, the concentration of toxicants or metabolites in urine may fluctuate. Creatinine correction is the most commonly accepted method for normalizing urine metabolite concentrations in order to reduce variability for first morning void urine (Barr and Needham, 2002). Metabolite results are considered questionable for samples with creatinine < 0.3 or > 3.0 g/L (O' Rourke et al, 2000).

2.6 Children's Pesticide Exposure Studies

Several researchers have established the studies of children's pesticide exposure involved with environmental monitoring.

Simcox *et al.* (1995) confirmed the theory that relatively non-persistent chemicals such as OP pesticides can be stable in residences. They found that housedust in agricultural area have high levels of pesticides that are not readily degraded indoors by sun, rain and microbes. Housedust, thus, represents another important source of exposure, particularly for children living in agricultural communities of Washington State.

Lu *et al.* (2000) studied OP pesticide exposure of preschool children in an agricultural community in Washington State. They indicated that the contamination can come either from the applications of OP pesticide indoor or outdoor environments for pest control purposes, from agricultural spraying. The results also reported that the residues of agricultural pesticides were detected on the work boots, steering wheels and children's hands of many agricultural families. A further study investigated the exposure through multiple pathways, indoor air, drinking water, soil, housedust, and

hand and toy wipes, and diets. It suggested that there were a difference of exposure pathways between children living in agricultural and non-agricultural regions (Lu *et al.*, 2004).

Curl *et al.* (2002) supported a previous study of Lu *et al.* (2000) by consistent the theory of a take-home or para-occupational exposure pathway. They found that agricultural chemicals could be moved from the workplace to residential environments through the activities of farm workers, thus preschool children of agricultural workers tended to receive greater pesticide exposure than other children because of farm proximity and their parent occupation.

Quandt *et al.* (2004) determined pesticides in wipe samples from floordust, toys, and children's hands of farmworker family household residences in North Carolina and Virginia. They found that agricultural pesticide detection was associated with housing adjacent to agricultural fields. They also suggested that the floor may be a reservoir of pesticide residues in the home, and presence of the pesticides on the floor predicted pesticides on hands or toys.

The findings of some studies in personal monitoring on children's skin related to the pesticide exposures were also presented below.

Camann *et al.* (1995) found that a total of 17 different pesticides, including agricultural herbicides such as atrazine, alachlor, 2,4-D, and dicamba, have been found on the hands of non-working children ranging from ages 3-15 years children on Midwestern and North Carolina farms.

Bradman *et al.* (1997) reported that two pesticides, diazinon and chlorpyrifos, were found on the hands of three out of five farmworker children sampled, at levels predicted by a screening risk assessment to result in exposures over the reference dose, whereas none of the children in non-farmworker homes had detectable pesticide residues on their hands.

Shalat *et al.* (2003) evaluated the correlation of OP pesticide loading on children's hands, housedust, and urinary OP metabolite in young children in south Texas. The preliminary findings suggested that surface loadings of pesticides on hands (ND-13.40 nmol/100cm^2) were more highly correlated with urinary metabolite (3.2-257 nmol/mol creatinine) than levels of pesticides in housedust (ND-78.03 nmol/100 cm^2).

Several studies have focused on biological monitoring of children's exposure to OP pesticides as follow.

Loewenherz *et al.* (1997) monitored increased risk of pesticide exposure for children less than 6 years of age living with pesticide applicators in Washington State. They found that DMTP was the dominant metabolite detected from children's urine, and was significantly higher in applicator children than in the reference. The results indicated that applicator children experienced higher OP pesticide exposures than did reference in the same community and that proximity to spraying is an important contributor to such exposure.

Azaroff (1999) investigated the phenomenon of non-occupational pesticide exposure among farmers' families in rural El Salvador through analysis of urine samples for alkylphosphate metabolites of OPs. The results indicated that children were exposed to pesticides through environmental as well as occupational routes during the planting season.

Aprea *et al.* (2000) evaluated six OP metabolites in urine of 195 children within 6 to 7 years of age, who lived in the municipality of Siena (Tuscany, Italy) by using biomonitoring technique. They found detectable concentrations DMP and DMTP in most of the detected in samples (96 and 94%, respectively). The urinary excretion of alkylphosphates in children was significantly higher than in adult residing in the same province.

Fenske *et al.* (2000a) estimated individual OP pesticide doses from urinary metabolite concentrations with a deterministic steady state model. They found that 56% of the dose estimated for children with agricultural workers exceeded the US EPA reference dose during the spray season. The finding indicated that children living in agricultural regions represent an important subpopulation in public health evaluation, and that their exposures fall within a range of regulatory concern.

Lu *et al.* (2001) assessed OP pesticide exposure among 110 children ages 2-5 years living in two Seattle metropolitan communities by measuring six DAP urinary metabolites. The results indicated that no significant differences in DAP concentration related to season, community, sex, age, family income, or housing type. Simultaneously, Fenske *et al.* (2001) suggested that biological monitoring could provide useful estimates of pesticide exposure among young children and indicated that children's exposure could differ regionally, and that young children may have higher exposures than the general population.

Heudorf and Angerer (2001) measured OP metabolites in urine samples from inhabitants of the former U.S. housing estate in Frankfurt, Germany. No evidence was found of increased internal exposure due to former chlorpyrifos application in the homes, either in children or adults.

Mills and Zahm (2001) found some OP metabolites (DMP, DMTP, and DETP) more often in urine of farmworker children than of their parents. The most frequently detected metabolite, DMP was found among 44% of children and 33% of adults. DMTP was detected among 33% of the children and 28% of the adults.

Koch *et al.* (2002) examined OP pesticide exposures of 2-5 years children in an agricultural community over an entire year in Washington State. They analyzed DAP metabolite in children's urine over an entire year. This study reported the temporal pattern of pesticide exposure and indicated that pesticide spraying in an agricultural region can increase children's exposure in the absence of parental work contact with pesticides or household proximity to farmland.

2.7 Intake and Dose Estimation

Once exposure point concentrations in all media of concern have been estimated, the intakes and/or doses to potentially exposed populations need to be determined. The absorbed dose differs significantly from the externally applied dose (called exposure or intake). Intakes and doses are normally calculated in the same step of the exposure assessment, where the former multiplied by an absorption factor yields the latter value (Asante-Duah, 1993). For non-carcinogenic effect, risk assessments consider the period of time over which the exposure occurred. Average exposures or dose over the period of exposure are sufficient for the assessment. These averages are general in the form of average daily dose (ADD). ADD can be calculated by averaging the potential dose (D_{pot}) over body weight and an averaging time (US EPA, 1992).

The general equation for calculating chemical intake (or ADD) by the population at risk is expressed by the following relationship:

$$I = (C \times CR \times CF \times FI \times ABS \times EF \times ED) / (BW \times AT) \quad (1)$$

Where:
- I = intake, adjusted for absorption (mg/kg/day)
- C = chemical concentration in media of concern (e.g., mg/L)
- CR = contact rate (e.g., mg soil/day; liters water/day)
- CF = conversion factor
- FI = fraction of intake from contaminated source
- ABS = bioavailability/absorption factor (%)
- EF = exposure frequency (d/years)
- ED = exposure duration (years)
- BW = body weight (kg)
- AT = averaging time (period over which exposure is averaged, days)

The quantity of a chemical absorbed into the bloodstream per event, represent by dose. When the level of dose from an intake is unknown, or cannot be estimated by a defensible argument, intake and dose are considered to be the same.

2.8 Risk Characterization

Risk characterization (or risk estimation as it is also known) is the process of estimating the probable incidence of adverse effects to receptors under various exposure conditions. The risk estimation involves the integration of the first three components of the risk assessment process, which are hazard identification, dose-response assessment, and exposure assessment.

Risk characterization may be subdivided into 4 different steps (Yassi, 2001): as indicated following :

Step	*Description*
1. Exposure	Pollutant concentration × exposure duration
2. Dose	Exposure (1) × dosimetry factors (absorption rate, consumption rate,etc.) divided with body weight or surface area
3. Lifetime individual risk	Dose (2) × risk characterization factor (carcinogenic potency, non-carcinogenic threshold) or severity, with uncertainty factors
4. Risk to exposed population	Individual risk (3) × number in exposed population (this should take into consideration age, and other susceptibility factors, population activities, etc.)

The risk to potentially exposed populations from exposure and subsequent intake of the chemicals of potential concern are characterized by the calculation of non-carcinogenic hazard quotients and hazard indices and/or carcinogenic risk. These parameters are then compared with applicable standards for risk decisions in hazardous waste management (Asante-Duah, 1993).

Non-Carcinogenic Risk Estimation

The overall potential non-carcinogenic hazard caused by the non-carcinogenic compounds is generally expressed based on the hazard index (HI). The non-carcinogenic effects are calculated according to the following relationship (US EPA., 1989a):

$$\text{Hazard Quotient (HQ)} = \text{Exposure} / \text{RfD}$$

Where: Exposure = chemical exposure level, or intake (mg/kg/day)
RfD = reference dose (mg/kg/day)

The sum of the hazard quotients for all chemicals of concern provide the hazard index for a given exposure pathway. The hazard index is given by:

$$\text{Hazard Index (HI)} = \Sigma\ (\text{HQ})$$

The exposure values can be estimated from the previous equation for average daily dose (ADD) calculation, whereas the RfD values are obtained from EPA's databases (e.g. IRIS). RfD have been established by the US EPA as thresholds of exposure to toxic substance less than the level which there should be no adverse health effects.

Hazard Indices Interpretation

According to US EPA guidelines on the interpretation of hazard indices (US EPA., 1989a), for any given substance there may be potential for adverse health effect if the hazard index exceeds unity (HI > 1), whereas a reference value of HI less than or equal to 1 should be taken as the acceptable reference or standard. For HI values greater than the unity, the higher the value, the greater adverse non-carcinogenic health impacts.