Risk Estimation Models Derived from Metabolic and Damage Parameter Variation in a Population

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ABSTRACT

Principles of estimation of dose-response relationships are discussed. Particular emphasis is given to the distribution within a population of parameters describing the metabolic processes governing the flow of a toxic substance through the body and the sensitivity of an organ to the substance considered. This approach should provide a means to identify the fraction of a population sensitive to low level exposures that could not be identified epidemiologically.

Concepts and assumptions necessary to set up general metabolic models are defined and discussed and so are models describing the occurrence of damage. Conditions applicable to individuals are extended to populations and a risk model is derived using distributions of the parameters in the population. Finally, risk estimation models which have been used for specific substances (methyl-mercury and cadmium) are analysed in relation to the proposed approach.

1 INTRODUCTION

Principles and methods for evaluating the toxicity of chemicals have been discussed in a WHO publication (WHO, 1978) and quantitative risk models are reviewed by several participants of the present workshop (Anderson; Cantor; Janyševa et al.; Bass; Day; Peto; and Hoel: this volume). Most of the models discussed in these papers are related to effects that result from interaction with genetic material of the cell. Our paper deals with some aspects of such effects but it is mainly concerned with effects that result from interaction of chemicals with other cellular targets.

Adverse effects of chemicals occur when a sufficient amount of the substance reaches sensitive targets in human tissues. An estimate of the risk may be obtained through a quantitative description of the metabolic behaviour of the chemical and of parameters relevant to tissue sensitivity. General principles of risk estimation are discussed in this paper and illustrated by two examples.
2 METABOLIC MODEL

A metabolic (pharmacokinetic or chemobiokinetic) model describes the flow of a substance from exposure medium through organs and tissues in the body to its elimination (for a historical review of such models and their application see, for example, Teorell, 1937a,b; Levy and Gibaldi, 1972; Task Group on Metal Accumulation, 1973; WHO, 1978). Some concepts and assumptions used in metabolic models are discussed in this section, indicating their applicability.

2.1 Uptake and Distribution

The kinetics of an exogenous substance in the body are described by its flow through a network of compartments, ranging from uptake to elimination. A compartment is defined as a part of the body (tissues or organs) in which the concentration of a substance behaves uniformly over time. Concentrations in subunits of a compartment may be different, but the concentration ratios between subunits remain the same at all times.

In every compartment, there are two major processes:

(1) the input of a substance or its metabolites either directly from the environment, or as a result of transport from another compartment; and

(2) the output of the substance by direct transport or biotransformation.

The input and output flows are time-dependent. In the lungs or the gastrointestinal tract (front end compartments), the input is determined by exposure. This input flow may be called absorbed dose rate. For any period of time, from $t_0$ to $t_1$, the following condition exists in a given compartment:

Content at $t_0 +$ accumulated input between $t_0$ and $t_1 =$ accumulated output between $t_0$ and $t_1 +$ content at $t_1$.

In most but not all instances, it is justified to assume that the flow of a substance from one compartment to another is approximately proportional to the concentration gradient, i.e. the net flow rate is proportional to the difference in concentration in two adjacent compartments. Using this assumption one can set up an equation linking the change of concentration in one compartment to that in another. One particular consequence of proportionality to the concentration gradient is an equation that describes the transfer of a substance from one compartment with an initial concentration $c_0$ and no further input, to another compartment which has always a low concentration maintained by enzymatic detoxication or excretion. Under such conditions the concentration $c_t$ in the first compartment or organ may be expressed as $c_t = c_0 \times \exp(-bt)$ where $t$ is time and $b$ is the elimination constant.

Another consequence of proportionality to the concentration gradient is the
steady state. Assuming a constant input, the internal concentration as well as the output will eventually reach a steady state in a compartment with output being equal to input and, consequently, the concentration equal to input divided by the elimination constant.

One major consideration in modelling kinetics of a chemical compound is the extent of compartmentalization that may occur. Substances that are readily absorbed from the site of application and are widely distributed in the body, behave kinetically in the same way as a substance that is distributed in total body water (for example, ethanol). For other substances, which are bound to macromolecules in membranes and tissues and which are very unevenly distributed in the body, the kinetics in various tissues are different. A compartment is frequently formed at the site of absorption but may involve other tissues of the body as well.

2.1.1 Compartments at the Site of Absorption

If substantial quantities of chemicals are retained in respiratory tissues after inhalation or in the mucosa of the gastrointestinal tract after ingestion, it is justified to consider these tissues as separate compartments. The uptake of gases and vapours (for reviews see Task Group on Lung Dynamics, 1966; WHO, 1978; Camner et al. 1979) depends on their solubility in water. Modelling of pulmonary tissue concentrations is important for gases like SO₂ that have a direct toxic effect on these tissues.

When an aerosol is inhaled, particles are deposited at various levels of the tracheobronchial and pulmonary tracts depending on breathing characteristics and aerodynamic size of particles. Soluble particles are taken up regardless of their deposition site and the retention time in pulmonary tissues is usually short, i.e. there is no compartmentalization at the site of absorption. Less soluble particles deposited in the tracheobronchial tract may be removed by mucociliary movement and swallowed before they are dissolved. Particles of limited solubility, which are not translocated to the gastrointestinal tract, may be retained for long periods in pulmonary tissues, thus constituting a source (or compartment) that may give rise to a considerable tissue dose even a long time after exposure. Such retention in pulmonary tissues has been shown to be important for respiratory carcinogenicity of some compounds of chromium and nickel.

The mucosa of the gastrointestinal tract often behaves kinetically as a compartment which is important for ingestion of large doses of chemical substances. In acute oral poisoning, local retention of a chemical is frequently a prerequisite for damage to occur in the gastrointestinal tract. For additional information on the uptake via the gastrointestinal tract see WHO (1978) and Camner et al. (1979).
2.1.2 Compartments in other Organs and Tissues

The transfer of a chemical from the site of absorption and its distribution to various organs in the body usually involves transport by blood and uptake by other tissues. Such uptake is often mediated by the 'diffusible fraction' in blood plasma and interstitial and intercellular fluids. If the diffusion of a substance in body organs is more rapid than some other step in metabolic behaviour, organs between which such rapid diffusion occurs may be regarded as parts of one compartment.

Binding of chemical substances to molecules with selective penetration properties to body organs may sometimes be of importance for uptake and retention in a compartment. An example is the selective uptake and retention of metallothionein-bound cadmium in the kidney. The uptake process involves passage of metallothionein-bound cadmium across the brush border membrane of the renal tubule cell against a concentration gradient. Obviously, such processes cannot be modelled by differential equations based on the concentration gradient. A rapid and almost complete uptake of cadmium-metallothionein from blood plasma in the renal tubule is assumed in the multicompartment model of cadmium metabolism discussed in section 5. This assumption is based on experimental evidence (Nordberg and Nordberg, 1975).

The rate of biotransformation of a substance and the permeability of cell membranes to that substance as it occurs in plasma and intercellular fluid, may be important for compartmentalization. The uptake of mercury vapour, physically dissolved in blood, into the brain and its subsequent oxidation to divalent mercury with a very long retention time represents a mechanism implying compartmentalization (Nordberg and Serenius, 1969). This mechanism is of considerable significance, since the central nervous system may be affected after inhalation of mercury vapour.

The processes of excretion and elimination of a chemical from the body as a whole are frequently the rate-limiting steps in the metabolism, and the mechanisms governing these processes are often of decisive importance for metabolic modelling. The rationale for treating methylmercury metabolism as a one-compartment system, for example, is the demonstration that the distribution to body organs is considerably faster than the excretion from the body (Swedish Expert Group, 1971).

A kinetic model of metabolism of a substance frequently involves several compartments. The accumulation in an organ has the effect of dampening and delaying the variations in the input flow. If, for instance, several compartments with the same behaviour are connected in series, a step increase in the input dose rate will eventually transform into a slow, gradual increase. Similarly, a single dose administered to an individual will result in a levelled-out output from an organ with a substantial retention capacity (long biological half-time).

These two examples suggest that high transient concentrations will affect
3 DAMAGE MECHANISMS

Damage to an organ or a tissue is an adverse change in some normal, essential function. The damage may be reversible or irreversible and may occur soon after a sufficiently high exposure or after a long-term exposure. There are several fundamentally different biochemical mechanisms that determine how damage of various kinds comes about. A brief discussion of the main damage mechanisms and their relationships to the receptor theory originally developed in pharmacology by Clark (1937) is given in section 3.2.

3.1 Mathematical Modelling of Damage Mechanisms

The basic assumption of toxicological causality is that all the information necessary to determine the probability of damage in a cell or tissue A within a compartment P is contained in the behaviour of its concentration in P over some specified time up to the time under consideration.

3.1.1 The Strain Function

Subsequently, a more specific assumption is made that the influence of the time sequence of concentrations in a compartment can be expressed by a single number, \( d \). This number, \( d \), indicates that the presence of a toxic agent in the tissue has produced, with or without some delay, a deviation in the normal biochemical state of cells. This deviation is called 'strain' and it is assumed to be measurable. If the strain is too great, reversible or irreversible damage will occur.

If homoeostatic mechanisms act rapidly, one may envisage that the tissue strain behaves over time in the same way as the concentration of the toxic agent. In this case the strain would be suitably represented by the concentration. An example is methylmercury poisoning, in which an irreversible toxic effect seems not to occur unless the concentration in the central nervous system (as represented by the blood value or the body burden) exceeds a certain value on some occasion.

At the other extreme, if the agent causes a more lasting or even irreversible biochemical change which does not disappear rapidly even if the agent is no
longer present in the tissue, the appropriate function of concentration would be the mean value or a weighted mean value (giving more weight to recent concentrations), or a (possibly weighted) time integral.

The damage function for the effects of chronic exposure to SO$_2$, for example, might be expected to be adequately represented by a function of this basic type. Also, latent tissue changes due to exposure to radiation or other carcinogenic agents may be described in a similar way.

3.1.2 Damage Criteria

For the purpose of computation the damage criterion has to be formalized, assuming that the deviation from equilibrium at a certain time is adequately represented by the strain function.

Deterministic damage may be defined as follows:

If the strain at time $t$ exceeds a threshold value for a given individual, then either a damage occurs at time $t$ or, if damage is already present, it remains.

For threshold effects such as occur in methylmercury poisoning, the strain is equal to concentration.

For comparison, stochastic damage may be defined as follows:

There is a probability that a damage will occur in a (short) time interval that includes time $t$. In the first order of approximation, this probability is proportional to the time $t$ and to the strain.

For example, in radiology it is assumed that for small doses the probability for cancer expression is proportional to the dose received within a given time interval.

3.1.3 Critical Effect, Critical Organ and Critical Concentration

The terms ‘critical effect’, ‘critical organ’ and ‘critical concentration’ are often used in toxicology. In cases where the strain can be considered as proportional to the concentration of the chemical and the threshold damage criterion applies, the definitions commonly used (Task Group on Metal Toxicity, 1976) can be applied directly.

However, for toxic effects which are stochastic the simple definitions are no longer sufficient. The main reason is that the critical concentration of the toxic agent does not exist. Nevertheless, it may be possible to use the critical effect concept with a slight modification. The additional assumptions that are required to define the concepts of criticality in these cases shall not be considered here; however, it should be pointed out that restricted categories of exposure patterns should be treated in a specific manner.
3.2 Damage Mechanisms: Biological Considerations

A toxic substance in contact with cells or tissues interacts with various ligands. The interaction between an exogenous substance and the biochemical constituents of tissues may sometimes follow the principles of enzyme inhibition established by Michaelis and Menten (1913) and later developed in pharmacology as 'receptor theory' (Clark et al., 1933; Clark, 1937; Ariëns, 1954; Stephenson, 1956). If the number of molecules of a toxic substance that are bound to tissue ligands is small, the function of the cell is usually not affected. When a large number of molecules is bound, the magnitude of the effect depends on the function that the binding ligands perform. A biochemical change may directly precede a pathological change or dysfunction. Such biochemical changes or biochemical lesions were described by Peters (1967). The concentration of a chemical (for example, a metal compound) at which undesirable functional changes, reversible or irreversible, occur in the cell has been defined as the 'critical' concentration (Task Group on Metal Toxicity, 1976). Since the binding between a chemical and tissue ligands is sometimes reversible and since most cellular components can be replaced and have a certain turnover time, most functional changes are reversible. The receptor theory used in pharmacology (see, for example, Stephenson, 1956) can explain the quantitative relationship between the dose (or concentration) of a chemical and the magnitude of graded effect.

The number of ligands available in tissues varies between individuals in a population. The extent of ligand binding that can occur without an adverse effect also varies between individuals. Each individual has a defined number of such ligands at a defined point in time; this is the basis for an individual damage threshold (deterministic damage). An example is the interference of lead with amino laevulinic acid dehydratase (ALA-D). This enzyme has a considerable reserve capacity, and a pronounced inhibition is required to adversely influence the erythropoiesis. For most effects, such a 'threshold' concentration of a chemical may be defined for certain cell types. In any individual, the function of an organ is impaired when a certain concentration of the chemical is attained in the most sensitive organ. This 'critical effect' is an important point in the spectrum of various effects that may be induced by chemicals, since it defines the dividing line between adverse and non-adverse effects.

An exception to the principles discussed above is a non-repaired damage resulting from the interaction with DNA. Since the DNA molecule contains the genetic code, interaction with DNA may cause an irreversible change in cellular function. In contrast to many other cellular targets (such as enzymes which have a certain reserve capacity enabling them to tolerate a small number of toxic molecules), the interaction of DNA even with a single exogenous molecule can give rise to a change in the genetic code and thus cause an irreversible damage. It is possible, therefore, that for interaction with DNA a 'threshold' does not exist.
and that damage is due to a stochastic process (stochastic damage). This type of damage is not the main subject of the present paper and will not be further discussed. For a more detailed discussion of carcinogenesis and mutagenesis see, for example, WHO (1978), IARC (1980) and Hoel (this volume).

4 INDIVIDUAL EFFECT MODELS AND POPULATION RISK MODELS

Modelling of concentration of a toxic substance in a tissue or compartment was discussed in section 2. Section 3 dealt with effects due to a given time series of concentrations. Taken together, these two components of a model determine whether or not a damage occurs at a given exposure. Certainly, the explicit form of a combined mathematical model varies considerably in complexity, ranging from a closed statistical formula to a system simulation of metabolic and damage processes. In the present section, a brief outline will be given of general principles that may be applied in extending a dose—effect model for an individual to a dose—response model for a population. This extension requires additional data that describe inter-individual variation of parameters.

When considering a single individual, a deterministic model can tell us whether or not a toxic effect will appear. The outcome will depend not only on exposure but also on the individual's metabolic status and tissue sensitivity.

At low exposures, the individuals which are damaged are primarily those who have unfavourable metabolic parameters (for example, high retention in tissues, low excretion rate) combined with high tissue sensitivity. The statistical distribution of parameters is either known or can be estimated. For instance, the model for methylmercury contains an individual parameter for the biological half-time. The corresponding statistical distribution may be represented by a normal distribution. The distribution of the threshold body burden has been approximated by log-normal and Weibull models (see section 5).

For a given exposure, the probability of a specified damage in a randomly chosen individual can be in principle computed by integration of the joint probability distribution of parameters over the dose range in which these parameters are associated with damage according to the combined metabolic/damage model.

Thus, the distributions of individual parameters can be combined with each other and with the structure of the metabolic and damage models to yield estimates of damage probabilities at varying dose levels of the toxic substance. For methylmercury, the procedure for the computation of the probability is illustrated in Figure 1.

The dose—response models discussed in the preceding text are particularly useful when the risk depends on the interplay of several parameters so that unfavourable combinations will lead to harmful effects.
5 APPLICATION OF RISK MODELS

The principles reviewed in the previous sections have been used in estimating the risk of central nervous damage in humans from exposure to methylmercury and of kidney damage from exposure to cadmium. The validity of these models is discussed in sections 5.1 and 5.2.

5.1 Methylmercury

The uptake of methylmercury from the gastrointestinal tract is virtually complete. Methylmercury distribution in body organs has a specific pattern, but all organs behave kinetically in a similar way. Therefore, a one-compartment model is appropriate (Swedish Expert Group, 1971). As already mentioned, the toxicity of methylmercury to the central nervous system in an adult individual may be considered to depend on the peak concentration in the brain tissue, and, therefore, a threshold model applies. The biological half-time of the compound has been estimated in persons exposed to high dietary levels in Iraq. Analyses of consecutive hair segments permitted the calculation of the biological half-time in several persons, and the inter-individual variation was estimated by Shahristani and Shihab (1974). Threshold body burdens in adults for different symptoms were also estimated (Bakir et al., 1973). By assuming that in adult individuals biological half-time is independent of the threshold body burden, these data
could be used to derive the joint probability distribution of the individual model parameters. The principles illustrated in Figure 1 for risk calculation at various doses of long-term intake of methylmercury yield the complete model (Nordberg and Strangert, 1976). The resulting dose–response curve when using a log-normal distribution of tissue thresholds is shown in Figure 2.

Depending on the choice of distribution (log-normal or Weibull or some other distribution) for individual thresholds, somewhat different results may be obtained particularly in the lower part of the dose–response curve; but other uncertainties (for example, the background prevalence of symptoms) have been shown to be of similar or greater importance for risk estimation at low doses (Nordberg and Strangert, 1978). These estimates of risk apply to adverse effects on the central nervous system of adult humans associated with long-term intake via food.

If population exposure is considered, the most sensitive segment of the population should be protected. Effects of methylmercury on the brain of the newborn when the mother was exposed to methylmercury during pregnancy has long been recognized. Recently, quantitative data have been obtained (Clarkson, personal communication; Marsh et al., 1980) for the concentrations of mercury in the hair of mothers giving birth to children who were later shown to have a retarded development of the central nervous system function. It might be possible to use these data for the estimation of risk in a population of children, associated with low level oral intake of methylmercury by mothers, by applying similar models as those employed for the effects in adults.
5.2 Cadmium

The same approach using a metabolic model and critical concentrations of the toxic agent in the critical organ has also been applied to estimate the expected appearance of kidney dysfunction in the exposed group (Friberg et al., 1974; Kjellström, 1977; Kjellström and Nordberg, 1978; Nordberg and Kjellström, 1979). The first attempts of such calculations (Kjellström et al., 1971; Friberg et al., 1974) were limited to extremely long exposure periods (over 20 years) and constant exposure conditions; a one-compartment model was applied and a critical concentration of cadmium in the kidney cortex of approximately 200 \( \mu g/g \) was assumed. Using this method, approximate intakes required for the appearance of proteinuria in an unspecified proportion of an exposed population were calculated.

Assuming that the rate-limiting step is the excretion from the body and considering kidney cortex as a subunit of the total body compartment, the use of a one-compartment model for cadmium may be justified when very long, low-level exposures are considered and only crude estimates of risk of proteinuria are required. However, when modelling metabolism over such long time periods, it is important to take into account the age dependence of body and organ weights as well as the amount of food consumed when exposure occurs via food. Even when modelling metabolism over long periods of time, a one-compartment approach implies considerable uncertainties.

A multicompartment model of cadmium metabolism (Kjellström and Nordberg, 1978; Nordberg and Kjellström, 1979) may be used to model over shorter time periods not only the concentrations of cadmium in the kidney cortex but also the concentrations in other tissues, excretion in urine etc., and more precise estimates of risk of proteinuria may be obtained. The complex nature of this model makes it necessary to simulate the metabolic process by stepwise computation of the intake and excretion of cadmium between eight tissue compartments. Such a model in combination with assumptions of inter-individual variation in metabolism has been used to estimate dose–response relationships for cadmium proteinuria and to compare such estimates with actual epidemiological observations (Kjellström, 1977). Although there are still many difficulties and uncertainties when estimating inter-individual variations of the critical concentration in renal cortex that gives rise to proteinuria, data obtained by means of in vivo neutron activation (Roels et al., 1979; Ellis et al., 1980) have helped to improve the risk estimates. Data on inter-individual variation of threshold concentrations for damage (logistic adaptation of 10% prevalence at about 200 \( \mu g/g \) and 50% at 300 \( \mu g/g \)) can be combined with metabolic data and estimations of risk of proteinuria can be made using the principles outlined in section 4 of this paper. As shown in Figure 3, such calculations have been made (Holmberg et al., 1982) for an average gastrointestinal absorption of 4.8% in the population, yielding an increase of 5%
above background prevalence for beta-2-microglobulinuria (proteinuria) at a daily intake of 200 μg per day. Risk in persons with a higher gastrointestinal absorption (20%) was also estimated. Such a high gastrointestinal absorption may occur in persons with iron deficiency (Flanagan et al., 1978).

It is evident from Figure 3 that the risk of proteinuria at intake levels lower than approximately 200 μg per day increases by a factor much larger than the relative increase in cadmium absorption (from 4.8 to 20%). At 100 μg per day of cadmium intake, for example, the risk increases from about 1% to about 12%.

![Figure 3](image)

Figure 3  Risk of proteinuria associated with long-term oral intake of cadmium estimated by using a model derived from metabolic and damage parameter variation in a human population; in general, a gastrointestinal uptake of 4.8% has been assumed. Separate risk estimates are shown for groups with a higher absorption (20%) which may occur in persons with iron deficiency anaemia. Redrawn from Holmberg et al., 1982 and reproduced by permission of Statens Vattenfallsverk

This rapid increase of risk is related to the slope obtained in the combined curve derived from the assumption of (1) a log-normal distribution of metabolic variation and (2) a logistic distribution of tissue thresholds in the renal cortex. The empirical observations on which the assumptions of parameter distributions have been made are incomplete and alternative assumptions (for example, Weibull or normal distribution) about parameter distributions may be used. Such alternatives would yield a somewhat different shape of the resulting dose–response curve.
5.3 Concluding Remarks

The two examples, methylmercury and cadmium, illustrate the application of models in estimating exposure–response relationships. Both examples are concerned with deterministic or 'threshold' type of damage. However, when specific information on parameter distributions in a population is available for a stochastic damage, a similar approach might be attempted (see Hoel et al., 1975). The dose–response may have to be modified on the basis of information obtained in metabolic and kinetic studies (Hoel, this volume).

In both examples (methylmercury and cadmium) the models imply estimation of parameters determining inter-individual variation in tissue sensitivity and of metabolic parameters. Such an approach offers certain advantages over more conventional methods. The resulting dose–response curve has a similar shape as the curve suggested by Mantel and Bryan (1961) for low-dose extrapolation. However, in the approach suggested in the present paper the slope and the exact shape are determined by the information available for the compound under consideration.

Rather than identifying a very small proportion of individuals with symptoms, which is often very difficult in epidemiological practice, in some cases it may be possible to separate the factors involved in poisoning and to estimate the distribution of the corresponding parameters in the population. When such data can be obtained and applied to a toxicological model, the risk estimates are determined by values of parameters that deviate only moderately from the normal values. Also, there are many uncertainties in assuming a specific mathematical form of dose–response relationship for extrapolation from high doses to very low doses. Calculating the risk from several, more reliably estimated portions of separate parameter distributions should provide a better procedure for risk estimation.

6 REFERENCES


