TOXICOKINETICS AND SAFETY FACTORS IN RISK ASSESSMENT

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INTRODUCTION

Risk assessment (the use of the scientific base to define the health effects of exposure of individuals or populations to hazardous materials) has experienced a substantial growth and refinement in many countries around the world (Codex Alimentarius, 1997). Whenever a risk assessment is undertaken, uncertainties are recommended to be acknowledged and taken into account. Two types of scientific uncertainties include those related to data obtained from epidemiology and toxicology studies and those associated with the selection of appropriate models. Model uncertainties arise, when data are to be used under other sets of conditions or data can not be obtained using currently available methods (Rodricks, 1995). In this paper, I will address some issues related to uncertainties in our current understanding and practice in risk assessment of anticholinesterase pesticides. The "safety factor" approach in international organizations will be the basis of discussion from papers written by Lu et al. (1995).

The Application of Safety Factors in Pesticide Risk Assessment

The application of uncertainty factors has been the subject of many reviews (Weil, 1972; Dourson and Stara, 1983; Calabrese, 1985; Lu, 1985).

Presently, the ADI-based quantitative safety evaluations representing "zero-risk" policies for chemicals have been used by various international organizations involved in setting limits on human exposure to pesticides since 1961. Lu (1995) compared safety factors used in international organizations including the World Health Organization for "threshold toxicants". Such approach assumed that "threshold toxicants" would pose some types of health hazard if exposures exceeded a threshold level. To arrive at the acceptable daily intake for humans, safety factors are applied. The safety factors are intended to compensate for the likely greater susceptibility of humans compared to laboratory animals and to account for heterogeneity among large human population (Lu, 1995).

Recent Activities Related to Risk Assessment of Anticholinesterase and "Safety Factors"

In the recent Codex Alimentarius Commission session (Codex Alimentarius, 1997), a report related to the recent consultation on dietary exposure assessment was introduced (FAO/WHO, 1995). The Joint FAO/WHO consultation on guidelines for predicting intake of pesticide residues in 1995 had recommended that Joint FAO/WHO Expert Committee on Pesticide Residue (JMPR) should assess the potential for acute toxicity when evaluating all pesticides. That is, a no-observed-adverse-effect level (NOAEL) should be identified from the database relating to acute exposure, and an appropriate safety factor applied (FAO/WHO, 1995). Acute reference dose which are based on acetylcholine esterase inhibition have been assigned to some pesticides by the JMPR. The consultation on dietary exposure assessment has then described methods to calculate acute exposure from dietary intake (FAO/WHO, 1997). Prior to these, The core Assessment Group (CAG) of the Joint Meeting on Pesticides (JMP, 1994), on its first meeting, discussed the allocation of parts of ADI to various exposure due to occupational, environmental and dietary intake. However, up to date this attempt has not been fully substantiated.

Renwick (1995) discussed the use of an additional safety factor for nature of toxicity in the ADI. However, these have not been applied consistently in many advisory bodies. Review of interagency comparison of some safety factors used in the risk assessment of compounds where nature of toxicity had been considered important in the determination of the total uncertainty factor applied, discussed some of these endpoints (teratogenicity and non-genotoxic carcinogenicity) (Renwick, 1995).

Anticholinesterase Pesticides

The Asia-Pacific Region accounted for approximately 16% of the total world market for pesticides and many developing countries are steadily increasing their demand for imported chemicals, many of which are used in agriculture (Forget, 1990).

Pesticides which act as cholinesterase inhibitors
inhibit cholinesterase enzyme activity in the nervous system. There are different types of cholinesterase in the human body, which differ in their location in tissues, substrate affinity and physiological function (Jeyaratnam and Maroni, 1994). In the presence of these pesticides, acetylcholinesterase is inhibited and is no longer able to break down acetylcholine into choline and acetic acid.

The resulting accumulation of acetylcholine in the parasympathetic nerve synapses (muscarine like action), the motor end-plate (nicotine like action), and in the central nervous system is relating to symptoms seen in humans and other animals in acute poisoning (Jeyaratnam and Maroni, 1994).

Acute toxicity of an anticholinesterase compound is the result of its potency on the primary target, the absorption, distribution, metabolism, excretion and pharmacodynamics.

Toxicity of pesticides depends on the intrinsic properties the compound, its route of uptake and bioavailability. However, level of exposure during occupational exposure on the other hand, is mainly affected by the agricultural practice and pesticide formulations used (Krieger, 1995). Uncertainties, often arise from exposure assessment step in the risk assessment paradigm, especially during occupational exposure.

**Cholinesterase Inhibition**

The similarity among animals on the cellular level serves as the basis upon which scientists have extrapolated or inferred functions from one species to another (Calabrese, 1984). The fractional organ composition of the body is remarkably constant throughout the mammalian kingdom. Many biological characteristics, in fact more than 100 highly diverse biologic parameters are mathematical functions of body weight and very accurately predicted across species (Krasovskij, 1976 cited in Calabrese, 1984). The differences in the sensitivity of acetylcholine esterase to inhibition by various anticholinesterase pesticide types are well described. Species related differences in the preferential inhibition of acetylcholine esterase by series of phosphates have been reported and explained (Kemp and Wallace, 1990).

As illustrated in table 1 in appendix 1, the critical effects chosen by the World Health Organization for cholinesterase inhibitor type pesticides were often based on the acetyl cholinesterase inhibition in human (Dourson and Lu, 1995).

According to Dourson and Lu (1995) "acetyl cholinesterase inhibition is a sensitive, reversible, biochemical indicator of exposure which justifies the use of smaller safety factor in the opinion of WHO. In 1987, Maxwell et al. reported that the best multiple regression model for the in vivo rate of cholinesterase inhibition contained three independent variables namely blood flow, carboxyesterase and cholinesterase. These could account for 94% of the observed variation seen in cholinesterase-inhibition observed in liver, diaphragm, plasma, spleen, heart, intestine, kidney, brain, skeletal muscle and lung of rats exposed to 90 µg/kg soman, im. (Maxwell et al. 1987). Tissue blood flow, alone could explain 79% of the total variation found.

Simulation models such as physiologically based-pharmacokinetics models are usually relied upon to provide the prediction of "target organ dose" from the actual chemical exposure scenario. Differential perfusion of tissues and the tissue group lumping by their perfusion and/or partition characteristics; vascular rich-group, muscle group, fat group and vascular-poor group provides information which would otherwise be almost impossible to obtain. DFP-specific parameter values were used with the model to simulate pharmacokinetic data from mice and rats.

Model parameter values specific for DFP in human, tissue/blood partition coefficients enzymatic and nonenzymatic DFP hydrolysis rates, and biomolecular inhibition rate constants for target enzymes were scaled from rodent data or obtained from the literature. Good agreement was obtained between model predictions and human-exposure data on the inhibition of red blood all AChE and plasma butyryl cholinesterase after an intramuscular injection of 33 micrograms/kg. DFP and at 24 hr after acute doses of DFP (10-54 micrograms/kg), as well as for repeated DFP exposures.

However, at the present time, these types of simulated models do not describe toxicity which may occur from toxic compounds to blood vessels (Andersen, 1995).

**A Carbamate Insecticide Poisoning Case Study and Discussion of Uncertainty Issues.**

In a study in Thailand, targerine growers were identified by circumstantial evidence characterization as "probable" cases which had been exposed to 90% SP formulation of methomyl (Sinhaseni et al. 1993). Two cases of these growers had an increased LDH in the isozyme pattern (Sinhaseni, in press).

Earlier, Saiyed et al. 1992, reported the increase in total lactate dehydrogenase enzymes in plasma of spraymen applying methomyl for five days. Isozyme patterns have been used as markers for tissue injury. Lactate dehydrogenase is a cytoplasmic
enzyme which exists as five different isoenzymes with differing electrophoretic mobilities. Each isoenzyme is a tetramer composed of H (heart-type) and/or M (muscle-type).

We report here a preliminary result of plasma LDH enzymes increase of rats exposed to single oral dose of methomyl at 7 mg/kg. Significant increase in LDH₄ levels were found in day 3 but did not significantly increased on day 5. Reduction of spleen weight and reduction of splenocyte viability were detected in a dose-related manner since day 1 in rats exposed to methomyl single oral doses of 6 mg/kg and 8 mg/kg. N-acetyl cysteine, an oxygen free radical scavenger were protective against cell death in these experiments (Samatiwat and Sinhaseni, in press).

The mechanism which certain cells in the body are affected by toxicants to release LDH₄ are limited in literature. LDH₄ is reported to be present in spleen of rats, pulmonary vascular endothelium of rats (Schultz et al. 1994) and polymorphonuclear neutrophils of hamster lung (Beck et al. 1983).

Lactate Dehydrogenase activity and isozyme patterns in tissues and bronchoalveolar lavage fluid from rats treated with monocrotaline pyrrole have been studied (Schultz et al. 1994). The author evaluated total LDH activity and isozyme patterns in the tissues, cell lysates, sera and cell-free BALF of rats after treatment with MCTP to determine the source of increased LDH activity. The author suggested that the most probable source of increased LDH activity is consistent with a contribution from the pulmonary vascular endothelium, a source rich in LDH₄.

The effects of some other toxicants on endothelial cells have been recently reviewed (Boor et al. 1995; Shireman and Pearce, 1996). The activation of dopaminergic receptors by fenoldopam mesylate, a dopaminergic vasodilator has been reported (Bugelski et al., 1989). Overt endothelial cell changes also occurred in these cells after damage to medial smooth muscle cell in arteries ranging from 200-300 µm diameter in the renal vascular beds. Vasodilating factor is released by endothelial cell following stimulation by acetylcholine (Shireman and Pearce, 1996). The protective effect of N-acetyl cysteine on endothelial permeability alterations induced by bradykinin tend to be organ-dependent (Deng et al. 1996). In the same report, hemodynamic alterations were noted in various organ including spleen.

Endothelial cells, once thought to be an inert and passive barrier is currently known to have a strategic role in the control of vasomotor tone, white cell trafficking and the development of arteriosclerosis (Shireman and Pearce, 1996).

The immune suppression from pesticides may be particular significant in developing countries where infections diseases cause nearly half of all deaths (UNIDO, 1996).

While epidemiological studies are considered to carry the highest weight of evidence in hazard identification step, there is concern about their ability to identify many human hazards, especially the ones with complex etiology.

When one considers animal data in toxicological studies in hazard identification, animal models which are representative of humans are desired. The effects of fenoldopam mesylate found in rats are reported not to be found in monkeys and dogs (Bugelski et al. 1989). These are obvious examples of uncertainties which are related to toxicological data. When one proceeds to dose-effect relationship step; the ability of epidemiological studies to actually identify and quantify exposures which may cause the integrated response in difficult. In order to overcome the complexity of extrapolation from exposure scenario from animal to human to predict "target organ dose", one relies on toxicokinetic models such as physiologically-based pharmacokinetic modelling. However, the models currently available do not describe toxicity which may occur as a result of toxicant exposure (Andersen, 1995). An article written by Bevan and Henrion (1994) also stressed the importance of including mechanisms inherent to the circulation that influence or modify its primary constrictor and dilator response in the model commonly used in conceptualization of the circulation.

Summary

For risk assessment of anticholinesterase pesticides, acetylcholinesterase inhibition is a sensitive, reversible indicator of exposure. However, use of smaller factors when data are available in human may not be justified in some specific cases. Direct action of anticholinesterase on receptor sites at various cell types in different target organs may lead to the more severe nature of toxicity. At the present time, uncertainties exist due to our limited capability to clarify many human diseases with complex etiology. The toxicokinetic models available may not accommodate some type of toxicants which their mode of action involve haemodynamic change or vascular cell injury.

Anticholinesterase pesticides are still widely used in many asian countries to control a variety of pest species in agricultural practices. The physiology of the
effects of these agents are complex and appropriate uncertainty or "safety factors" are needed to be acknowledged and taken into account from total exposure.

Risk assessment of anticholinesterase agents is related to complex biological system and we will probably, never, at least in our lifetimes, know everything we would like to know to assess risk. We can only do our best with current information available. However, one should be extremely careful and holistic when applying these uncertainties in risk assessment of anticholine-esterase pesticides.

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