DETERMINATION OF GENOTOXIC AND SYSTEMIC EFFECTS OF PESTICIDE / COMBINATION:

IN VITRO AND IN VIVO SCREENING OF ENDOSULFAN

KRISHNA KUMAR MISHRA

DIVISION OF TOXICOLOGY

DEPARTMENT OF ZOOLOGY, FACULTY OF SCIENCE

THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA

VADODARA – 390 002, INDIA

Ph.D. Thesis

DECEMBER, 2008

CONCISE SUMMARY

Endosulfan is a chlorinated hydrocarbon insecticide and acaricide of the cyclodiene subgroup, which acts as a poison to a wide variety of insects and mites on contact. Although it may also be used as a wood preservative, it is used primarily on a wide variety of food crops including tea, coffee, fruits, and vegetables, as well as on rice, cereals, maize, sorghum or other grains. Formulations of Endosulfan include emulsifiable concentrate, wettable powder, ultra-low volume (ULV) liquid, and smoke tablets. It is compatible with many other pesticides and may be found in formulations with dimethoate, malathion, methomyl, monocrotophos, pirimicarb, triazophos, fenoprop, parathion, petroleum oils, and oxine-copper. However, it is not compatible with alkaline materials. Technical Endosulfan is made up of a mixture of two molecular forms (isomers) of Endosulfan, the alpha- and beta-isomers (Arrebola *et al.* 2001).

The agricultural use of Endosulfan is very diverse and it is being used with several other insecticides or used at different intervals pre and/or post plantation. However it is difficult to draw a distinct line between the effect of Endosulfan alone or in combination since Endosulfan is often being used with other pesticides to evoke desirable end result in different crop fields against diverse groups of pest population

Endosulfan is a non-systemic insecticide and acaricide with contact and stomach action. It is used in the control of sucking, chewing and boring insects and mites on a very wide range of crops, including fruit (including citrus), vines, olives, vegetables, ornamentals, potatoes, cucurbits, cotton, tea, coffee, rice, cereals, maize, sorghum, oilseed crops, hops, hazels, sugar cane, tobacco, alfalfa, mushrooms, forestry, glasshouse crops, etc. It also controls tsetse flies (Tomlin, 1994).

Factory workers involved in synthesis of Endosulfan, workers involved in formulating and dispensing Endosulfan and Public health workers involved in pest control are occupationally exposed populations. Accidental poisoning of children by Endosulfan stored in the home or garage, accidental exposure among formulating plant workers and suicide attempts have a high risk circumstance of poisoning. Additionally individuals with a history of convulsive disorders would be expected to be at increased risk from exposure (Mackison *et al.*, 1981).

In general, the doses of Endosulfan, involved in cases of poisoning have been poorly characterized. In a summary of case reports (Lehr, 1996), the lowest reported dose that resulted in death was 35 mg/kg body weight; deaths have also been reported after ingestion of 295 and 467 mg/kg body weight, within 1hour of ingestion in some cases. Intensive medical treatment within 1 hour was reported to be successful after ingestion of doses of 100 and 1000 mg/kg body weight. The clinical signs in these patients were consistent with those seen in laboratory animals, dominated by tonic clonic spasms. In a case where a dose of 1000 mg/kg body weight was ingested, neurological symptoms requiring anti-epileptic therapy was still required one year after exposure (IPCS, 1998a

In Kerala, India, Endosulfan has been linked to hundreds of deaths and disorders among cashew nut plantation workers and villagers (THANAL, 2001). In Kasaragod province, where aerial spraying of Endosulfan occurred for at least 15 years, alarmingly high levels of Endosulfan residues have been detected in the blood and breast milk of villagers and cancers and disorders of the reproductive and central nervous systems are very common. A survey of only 123 houses found 49 cancer cases, 43 psychiatric cases, 23 epileptics, 9 with congenital abnormalities and 23 with mental retardation (Joshi, 2001).

A further concern stems from the evidence that Endosulfan may cause mutagenic effects in humans if exposure is great enough; Endosulfan has been shown to be genotoxic to human cells under experimental conditions (Lu *et al.*, 2000).

The Spanish conclusions about genotoxicity of Endosulfan were presented at the Working Group on the Classification and Labelling of Dangerous Substances: Meeting on Pesticides-Health Effects (25-27 April 2001) as documents ECBI/11/01 and ECBI/11/01 Add. This evaluation had taken into account all studies that were included both in the monograph and in the first addendum (July 2001). It was concluded that although Endosulfan was non-mutagenic *in vitro* and *in vivo* for somatic cells, it could not be precluded its mutagenicity for germ cells. In this sense, the notifier was requested by the ECCO 102-Peer Review Meeting to address the significance of published studies showing genotoxicity to germ cells.

However, in the results of the historic data were rather ambiguous and contradictory and hence, a combination of *in vivo* and *in vitro* tests were planed for evaluating the genotoxic potency of Endosulfan.

The safety assessment of new chemical substances includes the requirement for an assessment of genotoxic potencial based on the following guidelines:

1. International Conference on Harmonisation guidelines for testing of pharmaceuticals,
2. EU Technical Guidance Document for testing of industrial chemicals,
3. German BfR overview of strategies for testing of industrial chemicals,
4. UK Committee on Mutagenicity Guideline for testing of chemicals,
5. Food and Drug administration (FDA) Redbook,
6. Updated Recommended Strategy for Testing Oxidative Hair Dye Substances for their potential Muatgenicity/Genotoxicity,
7. Recommended Mutagenicity/Genotoxicity Tests for the Safety Testing of Cosmetic Ingredients to be included in the Annexes Council Directive 76/768/EEC and
8. FDA Guidance for Industry recommended Approaches to Integration of Genetic Toxicology Study Results.

Many assay systems have been developed and introduced for safety assessment of chemicals. More than a half of them are *in vitro* assay systems, therefore we can say that the field of genotoxicity started from the alternatives of animal experiments. Although there are many kinds of assay systems but none can detect chemical genotoxicity. Assays are generally endpoint specific, so we usually use several assays in combination referred to as "battery".

In addition to the regulatory guidelines (*viz.* OECD, ICH etc.) various workshops have been organized by professional scientists on Genotoxicity Testing (IWGT), International Association of Environmental Mutagen Societies (IAES) and its workshops (International Conferences on Environmental Mutagens i.e. ICEM) have given the following recommendations which is summarized briefly:

1. Bacterial Tests
2. Mammalian Cell Gene mutation Tests
3. In Vitro chromosomal aberration Tests
4. Bone marrow micronucleus and chromosomal aberration tests
5. Unscheduled DNA synthesis tests and
6. Germ cell tests

In conjunction to the above mentioned tests following tests are performed in present research work. Two most important endpoints are "gene mutation" and "chromosomal aberration". This battery was proposed for pharmaceutical drugs in international harmonization. This also includes gene mutation and chromosomal aberration *in vitro*, and one *in vivo* assay.

The requirement and relevance of assay methodologies selected for determination of genotoxic and systemic effects of pesticide / combination: *in vitro and in vivo* screening of Endosulfan is based completely on regulatory requirement, environmental concerns and controversies related to its toxicity and genotoxicity. The gist of the results of the current study is as follows:

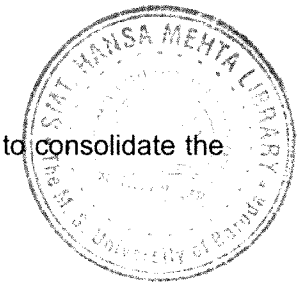
Endosulfan is mutagenic to bacterial test systems only in the absence of exogenous metabolic activation system. The mutagenicity was strong in case of *Salmonella* strain TA 102. Nonetheless, mutagenicity should be tested in sensitive strain like *Eschrichia coli* WP_2 uvrA with or without plasmids, since these strains are similar to *Salmonella* strains TA 102 in terms of sensitivity to mutate, for further confirmation.

Endosulfan was found to induce toxicity and clastogenicity in mammalian cells. The mutagenicity of Endosulfan is equivocal for mammalian cells. On one side it gives clear increase in clastogenic response and induces various chromosomal aberrations while on the other side somatic cell mutagenicity was not found in single trial of short term exposure. An experimental design including long term exposure with repeat experiments is required to detect mammalian cell mutagenicity as short term exposure may lead to death of the mutant cell (during 7 days expression period, CHO HGPRT assay) or expression period should be reduced to select mutant cells for expression. Therefore, confirmation on mutagenicity should be taken only after performing more sensitive test like mouse lymphoma assay (MLA).

Endosulfan is extremely toxic which leads to mortality on acute dosing and becomes clastogenic when applied repetitively both *in vitro* and *in vivo* tests.

Systemic exposure is all neurogenic type and clearly detected by increase in cholinesterase levels in serum. The hazards of Endosulfan is clearly due to targeting to the nervous system as is detected by the symptoms observed after exposure (all pertaining to nervous system) therefore, it is extremely toxic to the living system.

Endosulfan induces abnormality in sperm head and tail morphology. The anueniginecity of Endosulfan is very clearly detected by repetitive 28 days micronucleus test. This may be because Endosulfan takes time to get absorbed in to the mammalian system. The menace of Endosulfan induced malformations in human being could be related to its anuegenicity and gamete abnormality However, further carefully designed study on



reproductive and developmental toxicity needs to be undertaken in order to consolidate the current notion.

Recommendations

The indications are that Endosulfan poses serious risks to human health, especially under conditions of use in developing countries. Indeed, the chemical has been implicated in scores of cases of accidental death across the globe and long-term exposure has been linked to a range of serious disorders among villagers of southern India.

This pesticide kills indiscriminately, affecting not only pests, but also a range of other harmless or beneficial insects, with similar ramifications for species further up the food chain. Endosulfan's ability to harm is reflected in its mutagenic, aneugenic and systemic toxicity. This document presents ample evidence that Endosulfan might pose considerable risk to humans and the environment. In light of this the following recommendations are made:

Endosulfan is a highly dangerous, outdated chemical, the safe use of which cannot be guaranteed by many poor countries where it is still used. Governments should ban Endosulfan use, and Designated National Authorities in countries that are signatories of the Rotterdam Convention (India is party since 2005) should propose the chemical for inclusion in the Convention's Prior Informed Consent procedure. Endosulfan is already referred Chemical Review Committee (CRC 2) to be included in Annexure III. However, the decision is still pending.

Endosulfan is a persistent chemical that has been demonstrated to bioaccumulate in exposed organisms. As such, it should be included on the list of Persistent Organic Pollutants targeted for global elimination by the Stockholm Convention to further promote better practice. The World Health Organization should upgrade Endosulfan from Class II (Moderately Hazardous) to Class Ib (Highly Hazardous), in line with the USA's EPA classification. Such a move would assist many countries, which has banned all Class Ia and Ib chemicals, to promote safer agrochemical practices.

Safe alternatives to Endosulfan must be researched, identified and widely promoted. Pesticides Action Network Asia-Pacific lists a number of alternatives to Endosulfan use in different agricultural contexts. These include use of botanical pesticides (neem extracts) and parasitic wasps in rice production, and the use of baculoviruses, natural enemies and

pheromone traps to control cotton pests (Source: Environmental Justice Foundation Ltd. www.ejfoundation.org).

Ultimately, the action most ably protecting human and environmental health would be the withdrawal from sale of Endosulfan. This requires the agrochemical industry to rapidly phase out production of Endosulfan and to dispose of all stockpiles safely.