SYMPOSIUM PRESENTATION

Brazilian Experience with the Medium-Term Multi-Organ Bioassay: Scientific and Regulatory Developments

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Abstract

In 1996 the Brazilian Institute for the Environment (IBAMA) officially adopted a variation of the multi-organ initiation-promotion DMBDD bioassay as a valid source of evidence of the carcinogenic potential of pesticides. The protocol adopted by IBAMA was a modification of the one originally proposed by researchers led by Nobuyuki Ito, from the Nagoya City University Medical School. Among the modifications established in the Brazilian protocol were the use of both sexes of the outbreed Wistar strain of rats and two positive control test chemicals. The adoption of the modified DMBDD protocol was instrumental during the last decade for qualifying technical people and to spread knowledge on chemical carcinogenesis in Brazil.

Key Words: Two-year bioassay - mutagen/carcinogen screening - immunosuppression - estrogen - cell proliferation

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Introduction

Brazil does not have established a very strong industry for chemical development due to historical , political and economical reasons. Technical ingredients for the majority of manufactured chemicals sold in this country, such as pesticides, pharmaceuticals, food additives, cosmetics, etc., are imported from USA, EC and Asian countries, where the companies have their R&D facilities. Consequently, comprehensive expertise on pre-marketing and non-clinical toxicological studies has not been developed. Particularly, Brazil has not been able to run the standard long-term bioassay for carcinogenesis, due to the lack of skilled manpower and suitable animal strains and facilities.

Medium-term Testing in Brazil

Trying to overcome these limitations, in 1996 the Brazilian Institute for the Environment (IBAMA) officially adopted the alternative/complementary initiation-promotion DMBDD bioassay as a valid source of evidence for carcinogenic potential of pesticides (Brazilian Institute of Environment and Renewable Natural Resources, 1996). The reasoning for this strategic decision was that the adoption of a shorter, relatively less complex and less expensive bioassay could improve the expertise on chemical carcinogen identification, contributing to a more effective and consensual government regulatory action. The protocol adopted by IBAMA is a modification of the one originally proposed by researchers led by Nobuyuki Ito at Nagoya City University Medical School. The acronym DMBDD stands for the chemicals utilized to initiate multi-organ carcinogenesis: N-diethylnitrosamine (DEN), N-methyl-N-nitrosourea (MNU), N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN), N-N'-dimethylhydrazine (DMH), dihydroxy-di-N-propylnitrosamine (DHPN). Among the modifications established in the Brazilian protocol are the use of both sexes of the outbreed Wistar strain of rats and two positive control test chemicals, the non-genotoxic sodium phenobarbital (PB) and the genotoxic 2-acetylaminofluorene (2-AAF), and an experimental period of 30 weeks (de Camargo et al., 1999). The outbreed Wistar strain was chosen instead of the original F344 strain due to the ease in acquiring this rat strain in the country.

In order to subsidize their registration process by the Brazilian government, six non-genotoxic pesticides were tested for carcinogenesis during the 1996-2004 period at the Department of Pathology, UNESP Medical School. Besides those projects under contract with agrichemical industries, studies to better understand the lesions and processes ongoing in Wistar rats were also developed (de Oliveira et al., 1999; Rodrigues et al., 2002; Franchi et al., 2003). These studies showed that although apparently resistant to the modified chemical carcinogenesis protocol, DMBDD-treated male and female Wistar rats develop high incidences of pre-neoplastic and neoplastic lesions, mostly in the liver, colon, kidney and urinary bladder, than their respective controls. A striking finding was an increased incidence of renal mesenchymal tumors in Wistar female

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rats exposed to DMBDD (Moreira et al., 2000). The enhanced carcinogenesis process seemed to be partially associated with transient dysfunction in the immunologic system (Spinardi et al., 1999; Spinardi-Barbisan et al., 2000; 2004a; 2004b). It was also verified by non-isotopic PCR-SSP that selected mutations of TP53, H-ras and K-ras did not occur in the livers of male rats (Castelli et al., 2002).

The adoption of the DMBDD protocol for detection of potential carcinogenic pesticides was instrumental during the previous decade for qualifying technical people and for spreading knowledge on chemical carcinogenesis in Brazil. Recently, however, contract companies have progressively retreated from using the model. Among the reasons for this step backwards was the fact that chemical companies need to have validated and harmonized protocols in order to submit their dossier for product registration in countries other than Brazil and the DMBDD protocol at that time was not officially recognized for that purpose. Besides, the Brazilian federal government imposed good laboratory practices (GLP) for laboratory activities intended to support technical legislation and also the need for certification of professionals involved with the bioassays. Currently, our laboratory is devising new strategies to maintain the interest of governmental agencies and academia in potential alternative carcinogenesis bioassays in order to overcome the dependence of this country on the know-how generated abroad and, mostly important, to be able to establish a sound evaluation process for chemical carcinogenic hazard and risk.

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