The Pharma Innovation Journal 2021; 10(9): 1832-1834

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(9): 1832-1834

www.thepharmajournal.com
Received: 04-07-2021
Accepted: 13-08-2021

Dabholkar Akshay Rajkumar
MVSc scholar,
Department of Veterinary Pharmacology and Toxicology, KVASU, Kerala, India

Bibu John Kariyil
Assistant Professor,
Department of Veterinary Pharmacology and Toxicology, Kerala Veterinary and Animal Sciences University, Kerala, India

Shashikant P Vaidya
Assistant Director and Head,
Department of Clinical Pathology, Haffkine Institute for Training, Research and Testing, Mumbai, Maharashtra, India

Suja Rani S
Assistant Professor,
Department of Veterinary Pharmacology and Toxicology, Kerala Veterinary and Animal Sciences University, Kerala, India

Nisha AR
Assistant Professor and Head,
Department of Veterinary Pharmacology and Toxicology, Kerala Veterinary and Animal Sciences University, Kerala, India

Dhanush Krishna B
Assistant Professor,
Department of Veterinary Pathology, Kerala Veterinary and Animal Sciences University, Kerala, India

Corresponding Author:
Bibu John Kariyil
Assistant Professor,
Department of Veterinary Pharmacology and Toxicology, Kerala Veterinary and Animal Sciences University, Kerala, India

In vitro evaluation of carcinogenic effect of carbosulfan

Dabholkar Akshay Rajkumar, Bibu John Kariyil, Shashikant P Vaidya, Suja Rani S, Nisha AR and Dhanush Krishna B

Abstract
Carbosulfan belonging to carbamate class of insecticides are commonly used in agricultural practices for soil, foliar and seed treatment. There is a paucity of data about the carcinogenic effects of carbosulfan. Hence the present study was envisaged to evaluate the carcinogenic effects of carbosulfan using cell transformation assay in BALB/c 3T3 clone A31 cell line. Carbosulfan treatment at 50 µg/mL showed time dependent transformation in BALB/c 3T3 clone A31 cells revealing its carcinogenic potential. The data thus obtained could be useful in elucidating the various health hazards in humans and animals due to the environmental exposure to carbosulfan.

Keywords: Carbosulfan, without metabolic activation, carcinogenic, cell line

Introduction
Carbosulfan belonging to carbamate class of insecticides are commonly used in agricultural practices for soil, foliar and seed treatment. It is mainly applied on potatoes, sugarbeet, rice, maize and citrus to control wide range of insects affecting these crops. It is highly used as an alternative against the pests which were uncontrolled through organo-chlorine or organo-phosphorus pesticides and has also been recommended for the management of pyrethroid-resistant mosquitoes.

In spite of its wide use in agricultural practices, there is a paucity of data on the toxicological profile of carbosulfan on human and animal health. Mammalian cell cultures are useful tools to test toxicity of chemicals *in vitro* which facilitate the screening of environmental pollutants, risk assessment and safety evaluation in a cost effective and rapid way. The BALB/c 3T3 clone A31 cell line originated from BALB/c 3T3 mouse is recommended for carcinogenesis studies because these cells are sensitive and stable for fast transformation. The use of this cell line will also serve as alternatives to laboratory animals for testing the potential toxicity of xenobiotics, chemical agents and their mixtures. Hence the present study was envisaged to evalaute carcinogenic effect of carbosulfan in BALB/c 3T3 clone A31 cell line. The toxicological data so generated could be very useful to elucidate the various health hazards due to the environmental exposure of carbosulfan and provide the database for effective risk-management.

Materials and Methods
Carcinogenic effect of carbosulfan was assessed using BALB/c 3T3 cell transformation assay in which the expression of transformed foci was observed (IARC/NCI/EPA Working Group, 1985). In this assay, 4×10^6 BALB/c 3T3 clone A31 cells were seeded into 6 well plate and these cells were exposed to carbosulfan, ethyl methanesulphonate (EMS) and 0.1 per cent absolute ethanol. Ethyl methanesulphonate (200 µg/mL) served as positive control and absolute ethanol served as solvent control. Cells were treated with carbosulfan at concentrations of 50, 250 and 500 µg/mL for a period of 72 h. On termination of exposure, the treatment medium was replaced with normal medium and the cultures were maintained for further four weeks receiving medium change twice per week. At the end of 4 weeks, the transformed foci were observed under inverted phase contrast microscope (EVOS XL CORE, Life technologies, India) and observations were recorded at 40X.

Results and Discussion

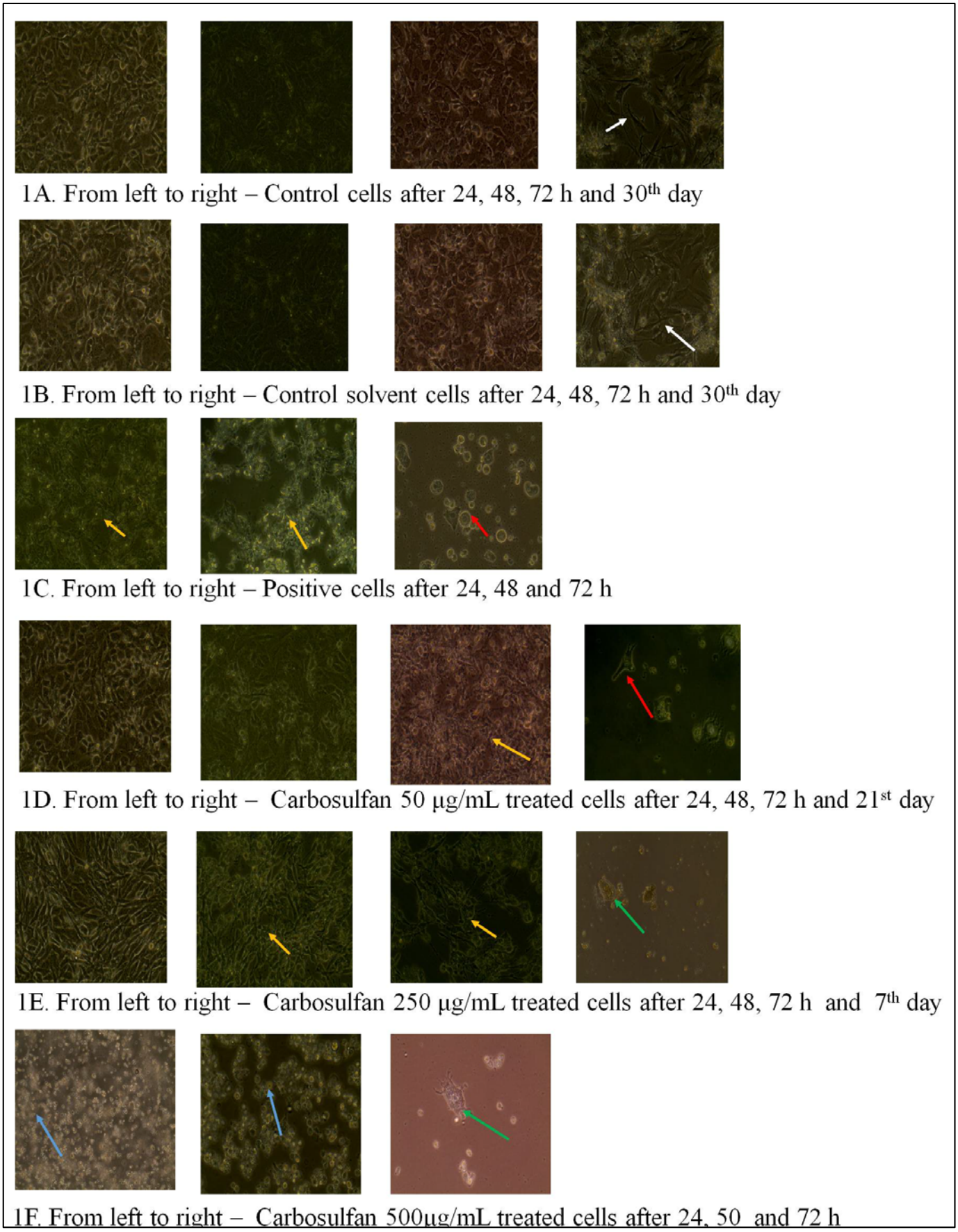

Plate 1: Cell transformation assay in BALB/c 3T3 clone A31 cell line exposed to various concentrations carbosulfan at 40X. White arrow – normal cells, yellow arrow – shrinkage and loss of cellular contact, red arrow – transformed cells, blue arrow – detached cells, green arrow – flattened cells.

In the present study, carbosulfan at 50 µg/mL induced cell transformation which was evident by spindle cells shaped on 21st day whereas, carbosulfan treatment at 250 and 500 µg/mL showed early detachment, loss of cells and flattened cells. The EMS treatment at 200 µg/mL also showed the transformation of BALB/c 3T3 cells at 72 h with the occurrence of transformed cells. Colacci *et al*. (1993) [2] reported that 1,1,2,2-tetrachloroethane (1,1,2,2- TTCE) at 500 µg/mL and 1000 µg/mL induced cell transformation in concentration dependent manner. Those transformed foci were noticed as small, fusiform, spindle shaped cells, randomly oriented at the focus edge. Similar kind foci were also observed with carbosulfan treatment at 50 µg/mL on 21st day. Mascolo *et al*. (2010) [3] reported transformation of BALB/c 3T3 cells into different shaped foci when exposed to various concentrations of ethinylestradiol, azathioprine and melphalan in concentration and time dependent manner. Perocco *et al*. (1995) [4] found that captan, captofol and folpet induced cytotoxicity and cellular transformation in BALB/c 3T3 cell line in the absence of metabolic activation. In the present study also, carbosulfan at higher concentration caused cytotoxicity and due to cytotoxic effect, most of cells were detached and the remaining cells showed cellular transformation without metabolic activation.

Hence the study concluded that carbosulfan could be considered as a potential carcinogenic agent. Further studies regarding the mechanism of toxicity are warranted. This data would be helpful in elucidating the various health hazards in humans and animals due to the environmental exposure to carbosulfan.

Acknowledgments

The first author and corresponding author duly acknowledge Kerala State Council for Science, Technology and Environment Student Project awarded to first author as Student Investigator and corresponding author as Principal Investigator. The financial assistance from Kerala Veterinary and Animal Sciences University as MVSc research grant to the first author is duly acknowledged. The facilities provided by Haffkine Institute for Training, Research and Testing, Mumbai for the successful conduct of the research work during the COVID-19 situation is duly acknowledged. The authors also acknowledge the facilities provided by Kerala Veterinary and Animal Sciences University, Pookode for the successful conduct of the research work.

References

1. IARC/NCI/EPA Working Group [International Agency for Research in Cancer/National Cancer Institute/Environmental Protection Agency Working group]. Cellular and molecular mechanisms of cell transformation and standardization of transformation assays of established cell lines for the prediction of carcinogenic chemicals: overview and recommended protocols. Cancer Res 1985;45:2395-2399.
2. Colacci A, Albini A, Melchiori A, Nanni P, Nicoletti G, Noonan D *et al*. Induction of a malignant phenotype in BALB/c 3T3 cells by 1, 1, 2, 2-tetrachloroethane. Int. J Oncol. 1993;2:937-945.
3. Mascolo MG, Perdichizzi S, Rotondo F, Morandi E, Guerrini A, Silingardi P *et al*. BALB/c 3T3 cell transformation assay for the prediction of carcinogenic potential of chemicals and environmental mixtures. Toxicol. *in vitro*, 2010;24:1292-1300.
4. Perocco P, Colacci A, Ciello CD, Grilli S. Transformation of BALB/c 3T3 cells *in vitro* by the fungicides captan, captafol and folpet. Japanese J Cancer Res 1995;86:941-947.