Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pesticide Biochemistry and Physiology 82 (2005) 103–114
PESTICIDE
Biochemistry & Physiology
www.elsevier.com/locate/ypest

In ovo embryotoxicity of α-endosulfan adversely influences liver and brain metabolism and the immune system in chickens

Pushpanjali [a], A.K. Pal [a,b], R.L. Prasad [a], A. Prasad [a], S.K. Singh [a], A. Kumar [a], S.B. Jadhao [b,*]

[a] *Department of Veterinary Biochemistry, Faculty of Veterinary Science and Animal Husbandry, Birsa Agricultural University, Ranchi 834 007, India*

[b] *Agricultural Research Service, Central Institute of Fisheries Education, 7 Bungalows, Versova, Mumbai 400 061(M.S.), India*

Received 27 July 2003; accepted 29 September 2004
Available online 7 April 2005

Abstract

There is a lack of laboratory-based embryonic chicken toxicity studies with the ecologically relevant low dose/s of endosulfan that utilizes a more practical approach such as the chorioallontoic membrane (CAM) injection. In this investigation, 2μg AR grade α-endosulfan/egg (40% of LD_{50} for embryos) was injected through the CAM in 12-day-old chicken embryos and the activities of glucose-6-phosphatase (G6Pase, EC 3.1.3.9), fructose 1,6-diphosphatase (FDPase, EC 3.1.3.11), adenosine triphosphatase (ATPase, EC 3.6.1.3) and succinic dehydrogenase (SDH, EC 1.3.99.1) and DNA and RNA content in liver and brain tissues and acetyl cholinesterase (AChE, EC 3.1.1.7) in the latter were determined at 24, 48, and 72 h post-exposure. The wet weight of the embryos did not differ between groups. Following endosulfan exposure, except increase in the hepatic ATPase activity ($P < 0.01$), there was a significant decrease in the following parameters: G6Pase activity in both the liver and brain ($P < 0.01$), SDH activity in the brain ($P < 0.01$), brain overall DNA and RNA concentration ($P < 0.05$), brain AChE activity ($P < 0.01$). Exposure of 18-day-old embryos to 2-μg endosulfan for 24 h caused decrease ($P < 0.01$) in the lymphocyte count and IgG content. Histopathology of thymus and bursa of Fabricius revealed a reduction in the population of thymic follicles, smaller thymocytes with the clear vacuoles in cytoplasm and fewer bursocytes accompanied by infiltration of erythrocytes in lymphoid follicles of the endosulfan-treated embryos. It was inferred that in ovo injection of 0.041 μg/g egg weight of α-endosulfan suppress gluconeogenesis (main energy source in embryonic life), nerve transmission, and immunity.

Keywords: Chicken embryo; Chorioallantoic membrane injection; Endosulfan; α-Isomer; Metabolic enzymes; Nucleic acids; Immunotoxicity

* Corresponding author. Fax: +91 22 26361573.
E-mail address: jsanju@hotmail.com (S.B. Jadhao).

0048-3575/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pestbp.2004.09.004

1. Introduction

There is an ever-growing incidence of organochlorine (OC) pesticide (e.g., endosulfan) poisoning in many countries of the world, which is attributed to extensive use of pesticides. In India, over 400 different types of pesticides are used [1] of which about 40% belong to the OC class of chemicals, such as aldrin, dieldrin, lindane, isodrin, heptachlor, and endosulfan. Average domestic use of endosulfan from 1987 to 1997 was 1.38 million pounds of active ingredient [2]. In 1999–2000, about 81,000 metric tones of endosulfan was manufactured in India [3]. Indiscriminate use of chlorinated hydrocarbons, like endosulfan has created a great concern for human, livestock and wildlife health as these chemicals pollute the environment, cause eco-system imbalances, and result in various biological disorders [4–6]. The organochlorine pesticides remain in the soil for many years and even for decades [7]. They are transferred from soil to edible crops and grasses and eventually to livestock and human beings through their upward movement in food chain. Residues are found in vegetables, grains, fruits, meat, and eggs [8]. Persistent organochlorines are distributed globally [9] including pristine polar and near polar locations [10].

Organochlorine pesticides remain for various reasons the main tool for combating disease vectors and pests in agricultural, animal husbandry, and health operations in many countries. Under commercial conditions, chickens are more vulnerable to pesticide toxicity in developing countries because poultry houses and the birds are dusted with pesticides. The sweepings and screenings from the government depots that are sold as poultry feed, after being declared unfit for human consumption, may be contaminated with pesticides [11]. Exposure of poultry to chemical pesticides poses health hazards to the birds themselves, which could result in economic losses, while at the same time posing a threat to public health due to the presence of residues in meat of the poultry. With regard to biology of wild birds under natural conditions, comparison of pre-pesticide era (1946) and recent years [12] clearly suggest that organochlorine pesticide accumulation in several wild birds and eggs is co-related with decline in wild birds population. This phenomenon has been observed in countries around the world.

Endosulfan, which is currently classified as Class II by WHO (moderately hazardous to human health) and category Ib (highly hazardous) by US EPA, has caused many tragedies in the world. Although, it is either banned or severely restricted in some countries, it is still widely used around the world [13]. In commercial preparations of endosulfan, the α- and β-isomer constitutes 70 and 30%, respectively. Under natural environment mixed pesticide use and toxicity is common. However, cases do exist where only a particular pesticide such as endosulfan is aerially spread over crops two to three times a year for more than 20 years [6]. In such instances, human and livestock toxicity to endosulfan is certain.

There is substantial evidence to suggest that endosulfan have immunosuppressive effects in avian [14–16] and mammalian species [1]. The presence of residual pesticides in eggs certainly has an impact on embryonic development. These impacts are manifested in a number of ways, such as reduced eggs shell weight and thickness [17], hatchability, poor growth, and less synthesis of immunogenic materials due to alteration of biochemical processes [8,18]. The alterations in the immune system during embryonic/fetal or infant development may be more dramatic and persistent than those from exposures later in life. There are no reports on endosulfan toxicity studies following injection in chorioallantoic membrane (CAM), which is more realistic and well-validated approach. CAM mediates gas exchanges with the extraembryonic environment until hatching and has a very thick capillary network. Also, there are many aspects of pesticide toxicity that remain unexplored and need attention for health and safety concerns. Embryogenesis in the chicken could serve as an excellent model to evaluate the in ovo effects of pesticides on metabolic pathways and histoarchitecture of different organs. In addition, the chicken embryo is highly sensitive to a broad spectrum of chemicals and physical agents and its development is closely related to mammalian morphological development [19]. The immune status of the intoxicated embryo may provide