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Evaluation of the Reproductive Toxicity of Diazinon in Male and Female Rat Offspring Exposed to Their Mothers Throughout Pregnancy and Lactation.

Diazinon is an organophosphate insecticide widely used in agriculture. It is also known to have adverse health effects. Possible reproductive toxic effects are less studied. The aim was to study the possible reproductive adverse effects of the diazinon on rat offspring exposed in utero and during lactation. Dams were gavaged with diazinon at 0, 10, 15 and 30 mg/kg/day prior to mating, during mating, pregnancy and lactation in separate groups. Maternal and reproductive outcome data and, male and female rat offspring reproductive parameter at puberty (PND70) and adulthood (PND 170) were examined. Male rat offspring were examined at puberty and adulthood for body weight, testis weight, epididymis weight, sperm count, motility and morphology, pituitary-gonadal hormone levels-FSH, LH, prolactin and testosterone, testicular marker enzymes activities-alkaline phosphatase, acid phosphatase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase and cholinesterase, qualitative and quantitative testicular and epididymal histology, total protein and Vitamin C levels and immunohistochemistry for 3 beta HSD. Similarly, female rat offspring were examined at puberty and adulthood for body weight, uterus weight, ovary weight, histological examination of ovary and uterus, pituitary-gonadal hormone levels, ovarian marker enzymes activities, ovarian total protein and Vitamin C levels and immunohistochemistry for 3 beta HSD. Diazinon caused a significant decrease in maternal body weight during gestation at 30mg/kg, but still there was an increase in body weight irrespective of the dose. The body weight, ovarian weight, uterus weight, plasma estradiol, prolactin and ovarian Vitamin C levels were decreased at postnatal day 22 in 30 mg/kg dose dam-groups. 30 mg/kg dose induced significant adverse effects both at puberty and at adulthood in rat offspring. 30mg/kg diazinon dose, the male rat offspring at puberty showed a decrease in testicular weight, sperm count, motility, with an increase in percent abnormal sperm, degenerative changes with a decline in pituitary-gonadal hormones, 3 beta HSD and total protein level. Moreover, an increase in activity of alkaline and acid phosphatase was also observed. At adulthood, there was a decrease in testicular weight, sperm count, motility with an increase in percent abnormal sperm and a decrease in pituitary hormones, 3 beta HSD and total protein levels with an increase in testicular marker enzyme levels. Female rat offspring at puberty in 30 mg/kg dose showed a decrease in body weight, ovarian weight, pituitary-gonadal hormones levels, 3 beta HSD, total protein and Vitamin C concentration and an increase in ovarian marker enzymes levels. At adulthood, the female rat offspring exhibited a decrease in body weight, ovarian weight, pituitary-gonadal hormone levels, 3 beta HSD, G6PD activity, total protein and Vitamin C concentration with an increase in the activity of alkaline phosphatase, acid phosphatase and lactate dehydrogenase. There was evidence of some adverse reproductive effects at 15 mg/kg dose in both male and female rat offspring. The study showed that most of the adverse effects were irreversible and were evident at both puberty and adulthood in rat offspring, although a few parameters reverted back to the normal growth pattern. The degree of toxicity of diazinon on the male and female rat offspring was identical at puberty and adulthood. Overall, diazinon is a reproductive toxicant in both male and female offspring when exposed during prenatal and postnatal life.

Key words: Reproductive toxicity, Diazinon, rat offspring
ABSTRAK

Diazinon merupakan sejenis racun serangga organofosfat yang telah banyak digunakan dalam pertanian. Racun serangga ini boleh mendatangkan kesan buruk terhadap kesihatan manusia. Walau bagaimanapun, kesan tosirknya kepada sistem pembiakan masih tidak banyak dikaji. Tujuan penyelidikan ini adalah untuk mengkaji kesan buruk diazinon ke atas pembiakan pada progeni tikus jantan dan betina pada peringkat pubert (PND70) dan dewasa (PND 70) telah dikaji. Progeni tikus jantan diberikan diazinon sebelum kelahiran dan berat badan sebelum dikeluarkan secara berasingan dalam kumpulan semasa musim pembiakan, kehamilan dan laktasi. Data keluaran maternal dan pembiakan serta parameter pembiakan untuk progeni tikus jantan dan betina pada peringkat pubert (PND70) dan dewasa (PND 70) telah dikaji. Progeni tikus jantan dikaji ke atas peringkat pertuban dan dewasa untuk berat badan, berat testis, berat epididimis, bilangan sperma, pergerakan dan morfologi, paras hormon FSH, LH, prolaktin dan testosteron, enzim fosfatase, asid fosfatase, laktat dehidrogenase, glukos-6-fosfatase dehidrogenase dan kolineresterase, histologi kualitatif dan kuantitatif testikel dan epididimis, jumlah protein dan paras Vitamin C dan ujian immuno kimia terhadap 3 beta HSD. Progeni tikus betina dewasa dan pubert pula dikaji: berat badan, berat uterus, berat ovari, ujian histologi terhadap ovari dan uterus, paras hormon pituitari-gonad, aktiviti enzim penanda ovari, paras hormon ovari, paras Vitamin C dan ujian immuno kimia terhadap 3 beta HSD. Diazinon menyebabkan pengurangan ketara berat badan maternal ketika bunting pada dos 30 mg/kg, tetapi masih ada peningkatan dalam berat badan dengan masa tanpa mengira paras dos. Berat badan, berat ovari, berat uterus, plasma estradiol, prolaktin dan paras Vitamin C menurun pada hari lahir yang ke 22 bagi kumpulan induk dengan dos 30 mg/kg. Dos 30 mg/kg memberikan kesan buruk pada kedua-dua puberti dan peringkat dewasa tikus. Didapati apabila dibandingkan dengan kumpulan kawalan, dos diberikan sebanyak 30 mg/kg memberikan kesan negatif yang nyata kepada kedua-dua kumpulan tikus jantan dan betina pada peringkat dewasa. Dalam progeni tikus pubert, berat testis, jumlah sperma dan motiliti, manakala peratus sperma abnormal dan penukaran atropik telah meningkat, disamping penurunan paras hormon pituitari-gonad, 3 beta HSD dan jumlah paras protein. Peningkatan dalam aktiviti alkali dan asid fosfatase juga diperhatikan, Pada peringkat dewasa, berat testis, bilangan sperma, motiliti didapati meningkat, manakala hormon pituitari, 3 beta HSD dan paras jumlah protein menurun diiringi dengan peningkatan paras enzim penanda testis. Progeni betina pada peringkat pubert dalam kumpulan dos 30 mg/kg menunjukkan penurunan dalam berat badan, berat ovari, paras hormon pituitari-gonadal, 3 beta HSD, jumlah protein dan kepekanan Vitamin C, dengan peningkatan paras enzim penanda ovari. Pada peringkat dewasa progeni tikus betina menunjukkan penurunan dalam berat badan, berat ovari, paras hormone pituitari-gonadal, 3 beta HSD, aktiviti G6PD, jumlah protein dan kepekanan Vitamin C disamping peningkatan aktiviti alkali fosfatase, asid fosfatase dan laktat dehidrogenase. Terdapat bukti menunjukkan kesan buruk pada pembiakan pada dos 15 mg/kg dalam kedua-dua progeni tikus jantan dan betina. Kajian ini juga menunjukkan kesan buruk tidak dapat diterbalikkan pada peringkat dewasa dan pubert dalam kedua-dua progeni tikus jantan dan betina. Walau bagaimanapun beberapa parameter dapat diterbalikkan Darjah ketoksikan diazinon pada progeni jantan dan betina adalah sama pada peringkat pubert dan dewasa. Secara keseluruhan, diazinon adalah toksik pada pembiakan dalam kedua-dua progeni jantan dan betina apabila didedahkan pada peringkat sebelum dan selepas kelahiran.

Perkataan kunci: Ketoksikan reproduktif, Diazinon, Anak tikus
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<table>
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<tr>
<td>3 β HSD</td>
<td>3 β-Hydroxysteroid dehydrogenase</td>
</tr>
<tr>
<td>AchE</td>
<td>Acetylcholinesterase</td>
</tr>
<tr>
<td>DBCP</td>
<td>1,2-Dibromo-3-chloropropane</td>
</tr>
<tr>
<td>DDT</td>
<td>Dichlorodiphenytrichloroethane</td>
</tr>
<tr>
<td>DZN</td>
<td>Diazinon</td>
</tr>
<tr>
<td>ELSIA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle Stimulating Hormone</td>
</tr>
<tr>
<td>G6PD</td>
<td>Glucose-6-Phosphate Dehydrogenase</td>
</tr>
<tr>
<td>GLC</td>
<td>Gas-Liquid Chromatography</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Haematoxylin and Eosin staining</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
</tr>
<tr>
<td>IMHP</td>
<td>2-isopropyl-4-methyl-6-hydroxypyrimidine</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate dehydrogenase</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinizing Hormone</td>
</tr>
<tr>
<td>NHMRC</td>
<td>National Health and Medical Research Council</td>
</tr>
<tr>
<td>NOAEL</td>
<td>No-observed-adverse-effect-level</td>
</tr>
<tr>
<td>OC</td>
<td>Organochlorine</td>
</tr>
<tr>
<td>OP</td>
<td>Organophosphate</td>
</tr>
<tr>
<td>OPIDN</td>
<td>Organophosphorus ester-induced delayed neuropathy</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
</tr>
<tr>
<td>PCBs</td>
<td>Polychlorinated biphenyls</td>
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</table>
PND Postnatal day
PON-1 Paraoxanase-1
STAR Steroidogenic acute regulatory protein
STD Seminiferous Tubular Diameter
TEPP Tetraethyl pyrophosphate
WHO World Health Organization
CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Pesticides are used in agriculture and public health to control insects, weeds, animals, and vectors of disease. The Food and Agriculture Organization of the United Nations (FAO) defined a pesticide as any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood, wood products or animal feedstuffs, or which may be administered to animals for the control of insects, mites, spider mites or other pests in or on their bodies (Anwar, 1997). Next to these intended effects, pesticides may also have adverse health effects for human beings. The main adverse health effects are difficulty in breathing, headache, neurological or psychological effects, irritation of skin and mucous membranes, skin disorders, effects on the immune system, cancer, and reproductive effects (Vainio, 1995; Al-Saleh, 1994). The manifestation of these effects depends on the type of pesticide and on level and duration of exposure.

Pesticides are also used in livestock production and public health programmes. However, liberal and indiscriminate use of pesticides has immense negative impact on the quality of environment and ultimately on the well being of animal and human population. The translocation of pesticides from soil and water, to plants and aquatic animals results in their entry into food chain and bioaccumulation (Rodrigo et al., 2001). Apart from this, higher living organisms were also directly exposed to the pesticides by way of inhalation and dermal absorption. Chronic exposure of humans and livestock to these xenobiotics resulted in various deleterious health hazards that are manifested over time. The resulting toxic effects on various systems of the body have a bearing on the quality of life in humans and the
Pesticides are occasionally used indiscriminately in large amounts causing environmental pollution, and therefore, are a cause of concern. Residual amounts of organophosphate (OP) and organochlorine (OC) pesticides have been detected in soils, water bodies, vegetables, grains and other foods products (John et al., 2001). OPs are known to cause inhibition of acetylcholinesterase activity in the target tissues (Kappers et al., 2001; Abu-Quare and Abou-Donia, 2001). Toxicities of OP pesticides cause adverse effects on many organs (Sultatos, 1994). Other systems that could be affected by OP intoxication are the immune system (Neishabouri et al., 2004; Masoud et al., 2003; Handy et al., 2002; McCauley et al., 2003), urinary system (Rodrigo et al., 2001), reproductive system (Joshi et al., 2003) and pancreas (Hagar et al., 2002). OPs could also cause haematological and biochemical changes (de Blaquiere et al., 2000).

There have been several studies conducted on organophosphate insecticides relating to the adverse reproductive effects on humans. Proven studies on organophosphate pesticides such as methyl parathion (De Silva et al., 2006; Prashanthi et al., 2006), paraoxon-methyl (Duquesne et al., 2006), dimethoate (Farag et al., 2006), chlorpyrifos (Ricceri et al., 2006; Aldridge et al., 2005a; Tian et al., 2005), malathion (Espinoza-Navarro and Bustos-Obregon, 2005; Eskenazi et al., 2004), diisopropyl methylphosphonate (DIMP) (Bucci et al., 2003) and methamidophos (de Castro et al., 2000a,b) showed adverse effects in male and female reproductive systems, with alterations in sexual behavior, decrease in fertility/sperm count, loss of the fetus during pregnancy, lactation as well as premature menopause being among the potential manifestations. These toxicants also interfered with the sexual functioning or reproductive ability of exposed individuals from puberty throughout adulthood.

Perusal of literature found limited information on the impact of pre and postnatal exposure to diazinon on the offspring's reproductive functions at puberty and adulthood (Cox, 2000). There are no reports on the adverse effects of diazinon on reproductive hormones and testicular/ovarian marker enzymes such as acid
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