RESEARCH ARTICLE

Received on: 20/12/2016
Published on:30/12/2016

Corresponding Author
AE Nantia
University of Bamenda, Faculty of Science, Department of Biochemistry, POBox 39, Bambili, Cameroon, (akonoed@yahoo.fr).

QR Code for Mobile users

Conflict of Interest: None Declared !

Effects of *in utero* exposure to chlorpyriphos-ethyl on male rat fertility

Augustave Kenfack[1], Akono Edouard Nantia[2*], Ferdinand Ngoula[1], Bertin Narcisse Vemo[1], Pierre Kamtchouing[3], Joseph Tchoumboue[1]

1 University of Dschang, Faculty of Agronomy and Agricultural Sciences, Department of Animal Production, POBOx: 222 Dschang, Cameroon

2 University of Bamenda, Faculty of Science, Department of Biochemistry, POBox 39, Bambili, Cameroon,

3 University of Yaoundé I, Faculty of Science, Department of Animal Biology and Physiology, POBox 812, Yaoundé, Cameroon

ABSTRACT

Background: Chlorpyriphos-ethyl (CE) is an organophosphate insecticide largely used in our communities for crop protection and hygienic purposes and limited data is available on its toxicity after prenatal exposure. In this study the effects CE were evaluated on the fertility in male rats exposed prenatally to the pesticide.

Methods: Four groups of primiparous pregnant rats were orally given every two days a dose of CE (0.00, 3.50, 4.25 or 10.50 $mg.kg^{-1}$ of body weight) for the whole gestational period. After delivery, male offspring were bred for 4 months without treatment and fifteen days to the end, each male was housed with 2 females for the fertility test. At the end of the experimental period, animals were weighed and then sacrificed and reproductive organs collected. The right cauda epididymis was used for the evaluation of sperm motility and concentration while the testis was submitted to histopathology analysis.

Results: No significant effect ($P > 0.05$) was shown on reproductive organ weights in rat born from CE-treated female as compared to the control group. The epididymal sperm count was comparable ($P > 0.05$) among treatments, but the highest dose of CE, 10.50$mg.kg^{-1}$ significantly decreased ($P < 0.05$) the percentage of motile sperms in male rats pups. This dose also induced an over spacing in animal testicular seminiferous tubules. The fertility rate was not significantly low in male rats prenatally exposed to 3.50$mg.kg^{-1}$.

Conclusion: These data suggest that *in utero* exposure of male rats to CE can lead to adverse effects such as decrease sperm motility with alteration of seminiferous tubules and slight reduced fertility.

Keywords: Chlorpyriphos-ethyl, fertility, *in utero* exposure, male rats, reproductive organs, sperm.

Introduction

The increase in agriculture production has gone simultaneously with more attack on crops from weeds and pests. This has prompted an increased use of chemicals including pesticides in order to prevent crop attack[1,2]. Extensive application of pesticides has allowed prevention or reduction of agricultural losses due to pests and promoted great availability of food[3]. From uses and applications pesticides can affect biological systems. In fact pesticides can enter the human and animalbody through inhalation, ingestion or dermal penetration[4].

Increased pesticides exposure has led to recrudescence of disorders associated to the chemicals including cancers, neurological disorders, reduced immunity, infertility and endocrine disorders[5,6]. The effects of these chemicals may be more serious on organisms from *in utero* or maternal exposure. In fact, such exposure have shown to cause neurobehavioral problems in infants, adverse birth outcomes, such as miscarriage, low birth weight and small head circumference[7,8].

Organophosphates are one of the highly represented classes of pesticides used in agriculture. In

Cameroon, the homologated organophosphates pesticides include Chlorpyriphos-ethyl (CE); a largely represented pesticide sold in different brand names such as Calloxyl C, Cyren 480 EC, Dursband 4E[9]. Its use is on a constant progress in Cameroon on a variety of crops including tubers, fruits and vegetables. As other organophosphates, CE may cause alteration of functions in pesticide users and/or consumers[10,11]. In fact, studies showed that chronic administration to male rats led to decrease of reproductive organs weight, epididymal sperm concentration and mobility[12,13]. It also increased thiobabituric acid levels and inhibited the activity of antioxidant enzymes superoxide dismutase and catalase in addition to the histopathological changes in the kidney of animals[14,15]. One of the very critical stages in animal development is maternal period. Exposure to any toxicant during this stage may lead to many disorders including neurological problems, leukemia, DNA damages, malformations, low birth weight[16-19]. Though studies have demonstrated the adverse effects of CE on animal functions including on antioxidant system and male reproductive function, no study has address the impact of this toxicant on animals through prenatal exposure. This study therefore aims to evaluate the effects of this insecticide on the fertility of male rats from maternal *in utero* exposure.

Materials and methods

Animals

Twenty four pregnant and primiparous wistar rats, aged four months, bred in Animal Physiology animal house of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang were housed in glass cages at room temperature with 12h-day light/dark cycle, with free access to feed and potable water. They were handled according to ethical guidelines of the Cameroon National Veterinary Laboratory.

Chemicals and instrumentation

The pesticide Chlorpyriphos-ethyl (CE) commercially named Pychlorex 48 EC from CHIMAC-AGRIPAR (Belgium) was used. The doses tested (3.50, 5.25 and 10.50 $mg.kg^{-1}$) were prepared by adding an appropriate volume of distilled water. The administered volumes were adjusted weekly to the body weight of animals.

The Mettler PE brand scale of capacity 160 g and precision 1 mg was used to weigh organs.

Study design

The adult female rats were randomly distributed into 4 groups of 6 animals each, namely one control and 3 experimental groups. The control animals received 0.583 $ml.kg^{-1}$ of distilled water while the experimental groups were treated with 3.50, 5.25 and 10.50 $mg.kg^{-1}$ of CE dissolved in the same volume of distilled water. These doses were defined from our previous studies[12,13]. Animals were orally treated every 2 days during the entire gestation period. After delivery the male pups were bred without chemical exposure for 4 months. Fifteen days to sacrifice, each rat was housed with 2 females to evaluate their fertility.

Data collection

The live weight of male offspring was measured at 4 months old. After sacrifice, the testes, epididymis, vas deferent, seminal vesicles and prostate were removed and weighed. The right cauda epididymis was weighed and minced in 100mL of 0.9% NaCl (36°C) for the evaluation of the sperm motility and concentration. To this end, the motile and non motile sperm cells were counted separately under the light microscope at magnification 400. The sperm concentration was estimated using the Thomas's haematocytometer.

The left testis was submitted to a histopathology analysis. Briefly, the entire testis was fixed in Bouin's fluid, washed dehydrated in alcohol baths of ascending grade, clarified in xylen immersion, hardened in paraffin, sectioned and stained with haematoxylin and eosin. The tissue sections were observed under a light microscope (400X) for any qualitative and quantitative changes in the seminiferous tubules and intertubular space.

Fertility rate in male rats was calculated on the basis of the number of males which procreated per lot. The obtained pups were examined for litter size, viability and sex ratio.

Statistical analysis

Results were expressed as mean ± standard deviation (SD). Differences between groups were assessed using one way ANOVA followed by the Duncan's test at the 5% significance. Analyses were performed using the SPSS 20.0 software.

Results

Reproductive organ weights

The weights of the testis, epididymis, seminal vesicle, vas deferent and prostate of the male pups exposed *in utero* to CE were not significantly different (P>0.05) from those of the control (Table 1).

Table 1: Reproductive organ weights of male rats

	Chlorpyriphos-ethyl (mg.kg^{-1})			
	0.00	3.50	5.25	10.50
Testes	0.44 ± 0.03	0.40 ± 0.04	0.43 ± 0.07	0.42 ± 0.04
Epididymis	0.16 ± 0.01	0.14 ± 0.03	0.15 ± 0.01	0.14 ± 0.02
Vas deferent	0.04 ± 0.01	0.04 ± 0.10	0.04 ± 0.01	0.03 ± 0.01
Seminal vesicle	0.33 ± 0.05	0.27 ± 0.09	0.36 ± 0.12	0.27 ± 0.06
Prostate	0.11 ± 0.04	0.10 ± 0.04	0.10 ± 0.01	0.12 ± 0.04

Data are in mean ± standard deviation of 6 observations

Epididymal sperm count and motility

The epididymal sperm number per cauda and per gram was comparable (P>0.05) among groups (Table 2). However, the percentage of motile sperms was reduced (P<0.05) at the highest dose of pesticide (10.50 mg.kg^{-1}) as compared to the other groups.

Table 2: The cauda epididymal sperm concentration and motility

	Chlorpyriphos-ethyl(mg.kg^{-1})			
	0.00	3.50	5.25	10.50
Number/cauda (x10^6)	54.93±8.08	73.33±6.80	65.73±12.69	49.60±24.57
Number/gram (x10^6)	344.31±54.43	528.00±78.00	495.01±136.65	351.89±167.0
Motility (%)	73.91±9.77	67.00±5.00	87.17±15.3	50.76±24.65[a]

a (P < 0.05) different from the other groups. Data are in mean ± standard deviation of 6 observations

Reproductive performances

The fertility rate was reduced (P<0.05) in males exposed prenatally to 3.50 and 5.25 mg.kg^{-1} of CE though it tend to increase with the dose 10.50 mg.kg^{-1} as compared to the control group (Table 3). The pup viability showed not significant dose-dependent decrease effect of CE, while the litter size and sex ratio did not vary between among groups.

Table 3: Reproductive performance of male rats

	Chlorpyriphos-ethyl (mg.kg^{-1})			
	0.00	3.50	5.25	10.50
Fertility rate (%)	75.00 ± 0.46	50.00 ± 0.53[a]	63.00 ± 0.52[a]	100.00 ± 0.00[a]
Litter size	6.00 ± 1.58	8.00 ± 2.24	5.00 ± 2.00	6.00 ± 4.20
Viability rate (%)	100.00 ± 0.00	100.00 ± 0.00	94.45 ± 9.62	83.50 ± 23.30
Sex-ratio	83.33 ± 16.67	74.74 ± 3.18	62.22 ± 20.37	75.00 ± 35.40

a (P < 0.05) different from the other groups. Data are in mean ± standard deviation of 6 observations

Histopathology

No abnormality was observed on the testis histological sections of the testis in male rats from control females and those treated with 3.50 and 5.25 mg.kg^{-1} of CE (Figure 1A, B and C). However, the 10.50 mg.kg^{-1} induced an over spacing of seminiferous tubules in the testis of perinatally exposed male pups (Figure 1D).

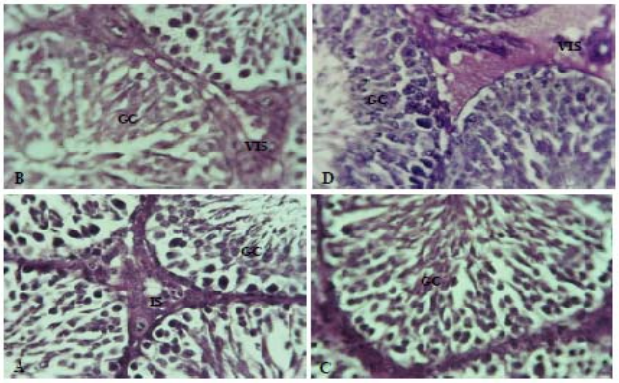

Figure 1: Histological sections of testis of rat exposed *in utero* to CE.

A : control vehicle, B : 3.50 mg.kg^{-1}, C : 5.25 mg.kg^{-1}, D : 10.50 mg.kg^{-1}. GC : germ cells, VIS : vacuolization in the interstitial space.

Discussion

The embryonic and foetal periods are very critical and sensitive stages for the developing organism. In fact any hormonal imbalance for example could result in significant change in the development of the conceptus. Moreover perinatal alteration could be latent and then be observed only late in the life[20-22]. Exposure to toxic environmental chemicals including pesticides have been associated with neurodevelopment impairment, cancers, decrease in fertility, semen quality, increase spontaneous abortion and fetal loss, impaired fetal growth, low birth weight, and preterm delivery, and congenital malformations[22-25].

In this study, gestational exposure of male pups to CE did not significantly affect their organ and body weight. Organ development is dependent on many factors including male hormone testosterone which plays a crucial role in guiding normal cell differentiation in early life forms[26]. Therefore exposure to endocrine disrupting substances in the egg or in the womb can substantially alter the normal process of development with consequence on organ and body weights. Mature animals may also be affected, but it is the developing organism that is especially vulnerable[27,28]. Similar to the present study, authors also showed the non influence of endocrine disruptor or hormone modulators on male organ and body weight after exposure through the placenta[29,30].

The testis has as one of the main functions the production of germ cells which undergo full maturation in the epididymis[31]. The epididymal journey is expected to confer to sperm cells full motility, a vital parameter for potential fertilization[32]. In the present study, CE treatment decreased sperm motility and the male rat fertility rate with a not significant dose-dependent decrease in pup viability. Any chemical that affects the sperm motility may have significant consequence on the male fertility[33]. In fact the fewer the number of sperm and the lower the motility, the lower the fertility of animals because limited spermatozoa would be available for impregnation of females[34]. In another study using post-natal exposure model, CE administration also reduced sperm motility[12,13]. These observations indicate sperm motility as one of the specific target of CE toxicity. It may also point out a certain specific toxicity of this subclass of organophosphorus pesticides on the male reproductive function, as a parent compound chlorpyrifos has shown alteration of germ cells with disruption of seminiferous tubules[35]. Alteration of sperm parameters in this study is consistent to the histological analysis of the testicular section that showed the degeneration of the seminiferous tubules with few germinal cells in the lumen. On the other hand the reduced sperm motility in pesticide exposed animals can be due to morphological alteration of sperm cells, which may lead to a reduce viability of pups[36].

In conclusion, *in uetro* exposure of male rats to CE induces damages of the seminiferous epithelium, decreases sperm motility and animal fertility with moderate effect on the pup viability rate. These findings suggest a certain contribution of CE in the infertility of animal and human populations exposed to such a chemical.

References:

[1] FAO. Fertilizers and their use, a pocket guide for extension officers. Fourth edition Food and Agriculture Organization of the United Nations, International Fertilizer Industry Association Rome, 2000.

[2]World Bank. Enhancing Agricultural Innovation: How to go Beyond the Strengthening of Research Systems. The International Bank for Reconstruction and Development. World Bank: Washington. DC. USA. 2007; p. 135.

[3] Cooper J, Dobson H. The benefits of pesticides to mankind and the environment. Crop Prot, 2007; 26 (9):1337-1348.

[4]Wafa T, Nadia K, Amel N, Ikbal C, Insaf T, Asma K, Hedi MA and Mohamed H. Oxidative stress, hematological and biochemical alterations in farmers exposed to pesticides. J. Environ. Sci. Health B., 2013; 48(12):1058-69.

[5]Abhilash PC, Singh N. Pesticide use and application: An Indian scenario. J. Hazard Mater, 2009; 165:1–12.

[6]Manfo TFP, Moundipa FP, Déchaud H, Tchana NA, Nantia AE, Zabot MT, Pugeat M. Effect of Agropesticides use on male reproductive function: A study on farmers in Djutitsa (Cameroon). Environ. Toxicol., 2012; 27(7):423-32.

[7] Pastore M, Hertz-Picciotto I, Beaumont JJ. Risk of stillbirth from occupational and residential exposures. Occup. Environ. Med., 1997; 54(6):511.

[8]Shen HQ, Main KM, Virtanen HE, Damggard IN, Haavisto A M, Kaleva M, Boisen KA, Schmidt IM, Chellakooty M, Ska kkebaek NE, Toppari J, Schramm KW. From mother to child: Investigation of prenatal and postnatal exposure to persiste nt bioaccumulating toxicants using breast milk and placenta biomonitoring. Chemos., 2007; 67(9): 256-262.

[9] MINADER. Liste des pesticides homologues au Cameroun au 31 juillet 2013, Liste réservée au Grand Public. Ministere de l'Agriculture Developpement Rural, 2013; 40p. http://www.minader.cm/uploads/File/Liste%20des%20Produits%20homologu%C3%A9s%20et%20APV%2031%20JUILLET%202013%20Grd%20Public%20V1.pdf Access 30 September 2016.

[10] Davies R, Ahmed G, Freer T. Chronic exposure to organophosphates: background and clinical picture. Adv Psychiatr Treat, 2000; 6:187–192.

[11] Blain GP. Adverse health effects after low level exposure to organophosphates. Occup. Environ. Med., 2001; 58:689-690. doi:10.1136/oem.58.11.689.

[12] Kenfack A, Ngoula N, Tchoumboué J, Kamtchouing P. Influence of chlorpiryphos-ethyl in some male reproductive parameters in albinos rats exposed post-natally. Int. J. Biol. Chem. Sci., 2007; 1: 237- 243.

[13] Kenfack A, Ngoula F, Dzeufiet WPD, Ngouateu BO, Tsambou MAM, Chombong KJ, Zeukeng ZMG, Nyuysemo LI, Jemima A , Nain PT, Kamtchouing P, Tchoumbou J, Vemo BN. Persistence of the reproductive toxicity of chlorpiryphos-ethyl in male Wistar rat. Asian Pac J Reprod, 2015; 4(1): 37-40.

[14]Gultekin F, Delibas N, Yasar S, Kilinc I. *In vivo* changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and vitamin E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats.Arch Toxicol., 2001; 75(2):88-96.

[15]Oncu M, Gultekin F, Karaöz E, Altuntas I, Delibas N. Nephrotoxicity in rats induced by chlorpryfos-ethyl and ameliorating effects of antioxidants.Hum. Exp. Toxicol., 2002; 21(4):223-30.

[16]Ward MH, Colt JS, Metayer C, Gunier RB, Lubin J, Crouse V, Nishioka MG, Reynolds P,Buffler PA.Residential exposure to polychlorinated biphenyls and organochlorine pesticides and risk of childhood leukemia. Environ. Health Perspect., 2009; 117: 1007-1013.

[17] Dordevic M, Vujic A, Jovanovic B, Dordevic G, Sazdanovic M, Sazdanović P. Neonatal outcome following exposure to organophosphorous pesticides. Arch. Biol. Sci., Belgrade 2010; 62: 1-8.

[18] Wanga L, Wu T, Liua X, Andersona LJ, Alamiana A, Fuc M, Lic J. Pesticide exposure during pregnancy and low birth weight. WHO South-East Asia. J. Public Health, 2012; 1(3):232-238.

[19] Shelton FJ, Geraghty ME, Tancredi JD, Delwiche DL, Schmidt JR, Ritz B, Hansen LR, Hertz IP. Neurodevelopmental Disorders and Prenatal Residential Proximity to Agricultural Pesticides: The CHARGE Study. Environ. Health Perspect, 2014 ; 1-29.

[20] Perera F, Herbstman J. Prenatal environmental exposures, epigenetics, and disease. Reprod Toxicol., 2011; 31(3): 363–373

[21] WHO. Possible developmental early effects of endocrine disrupters on child health. WHO Library Cataloguing-in-Publication Data, 2012; ISBN 978 92 4 150376 1.

[22] Di Renzo CG, Conry AJ, Blake J, DeFrancesco SM, DeNicola N, Martin Jr.NJ, McCue AK, Richmond D, Shah A, Sutton P, Woodruff JT, van der Poel ZS, Giudice CL. International Federation of Gynecology and Obstetrics opinion on reproductive health impacts of exposure to toxic environmental chemicals. Int J Gynaecol Obstet, 2015; xxx, xxx–xxx. http://www.figo.org/sites/default/files/uploads/News/Final%20PDF_8462.pdf. Date of access 30 September 2016.

[23] Bouchard FM, Chevrier J, Harley KG, Kogut K, Vedar M, Calderon N, Trujillo C, Johnson C, Bradman A, Barr BD, Eskenazi B. Prenatal Exposure to Organophosphate Pesticides and IQ in 7-Year-Old Children. Environ Health Perspect, 2011; 119:1189–1195. doi:10.1289/ehp.1003185.

[24] WHO. World Health Organization, United Nations Environment Programme. State of the science of endocrine disrupting chemicals - 2012. http://www.who.int/ceh/publications/ endocrine/en/. Published 2013. Accessed September 7, 2015.

[25] Asghar U, Malik MF, Javed A. Pesticide Exposure and Human Health: A Review. JEcosys Ecograph, 2016; S5: 005. doi: 10.4172/2157-7625.S5-005

[26] Rao KP, Burnett LA. Development of the Male Reproductive System. In: Clinical Urologic Endocrinology: Principles for Men's Health. Springer-Verlag London, P.K. Kavoussi et al. (eds.), 2013; DOI 10.1007, ISBN: 978-1-4471-4405-2, pp.11-24.

[27] WHO. Summary of Principles for Evaluating Health Risks in Children Associated with Exposure to Chemicals. World Health Organization 2011 ; Geneva, Switzerland, ISBN 978 92 4 150117 0.

[28] Ben Slima A, Ben Abdallah F, Keskes-Ammar L, Mallek Z, El Feki A, Gdoura R. Embryonic exposure to dimethoate and/or deltamethrin i mpairs sexual development and reproductive success in adult male offspring mice. Andrologia, 2012 ; 44(1):661-6.

[29] Turner KJ, Barlow NJ, Struve MF, Wallace DG, Gaido KW, Dorman DC, Foster PM. Effects of *in utero* exposure to the organophosphate insecticide fenitrothion on androgen-dependent reproductive development in the Crl: CD(SD)BR rat. Toxicol Sci., 2002 ; 68(1):174-83.

[30]Nantia AE, Manfo TFP, Beboy EN, Travert C, Carreau S, Monsees KT, Moundipa FP. Effect of methanol extract of *Basella alba* L. (Basellaceae) on the fecundity and testosterone level in male rats exposed to flutamide *in utero*. Andrologia, 2012; 44(1):38-45.

[31] Holstein A-F, Schulze W, Davidoff M. Understanding spermatogenesis is a prerequisite for treatment, Review. Reprod Biol Endocrinol, 2003; 1:1-16.

[32] Cornwall GA. New insights into epididymal biology and function. Hum Reprod Update, 2009; 15:213–227.

[33] Manfo TFP, Nantia AE, Mathur PP. Effect of Environmental Contaminants on Mammalian Testis. Curr Mol Pharmacol, 2014; 7: 119-135.

[34] Sharpe MR. Sperm counts and fertility in men: a rocky road ahead. EMBO reports, 2012; 13(5): 398-403.

[35] Joshi SC, Mathur R, Gulati N. Testicular toxicity of chlorpyrifos (an organophosphate pesticide) in albino rat. Toxicol. Ind. Health, 2007; 23: 439-444.

[36]Tarín JJ, Pérez-Albalá S, Cano A. Consequences on offspring of abnormal function in ageing gametes. Hum Reprod Update, 2000; 6(6): 532-49.