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ORIGINAL ARTICLE

Subchronic Toxicity of a Terbufos-based Pesticide (Counter 15FC) in Adult Male Rats

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KEYWORDS

Counter 15FC;
Neurological Dysfunction;
Oxidative stress;
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Toxicity

ABSTRACT: This study aimed at evaluating the subchronic adverse effects of Counter 15FC (a terbufos -based pesticide formulation) in adult male *Wistar albino* rats, focusing on neurological, liver, kidney and reproductive functions. Five groups of animals were administered either vehicle (Control) or Counter 15FC at doses 0.1 - 3 mg/kg body weight (bwt) for 9 weeks. All surviving animals were sacrificed at the end of the treatment period, and their liver, kidneys and reproductive organs weighed. Testosterone levels, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities, biomarkers for liver function (alanine transaminase and aspartate transaminase activities), kidney function (creatinine and uric acid) and total antioxidant capacity were assessed in serum. Oxidative stress markers (thiobarbituric acid reactive species, reduced glutathione levels and catalase activity) were determined in testicular and liver homogenates. Counter 15 FC at the dose of 3 mg/kg bwt induced tremors, seizures and death of 4 animals after 6 days of experiment. The pesticide formulation at 1 mg/kg bwt inhibited AChE and BuChE after 9 weeks. Moreover, the pesticide doses 0.1 and 0.3 mg/kg bwt inhibited testicular catalase activity, while other parameters investigated remained unchanged. Overall, results from this study suggest that exposure to Counter 15 FC can be fatal. The pesticide toxicity occurs at least in part through inhibition of cholinesterase and catalase activities in nervous system and testis, respectively.

INTRODUCTION

Pesticides are chemicals used to destroy, prevent or control activity of pests (unwanted microorganisms, plants or animals) that cause damage or interfere in the production, processing, storage or transport of food, animal feed, agricultural products, wood and wood products. Pesticides are also used for the control of insects, arachnids or other pests in animals [1]. However, previous studies have shown that pesticides cause adverse health effects in non-target species leading to neurological, reproductive, renal and hepatic dysfunctions [2-4]. One of these agrochemicals is Counter 15FC, a pesticide formulation used by farmers in Cameroon [5].

Counter 15FC is a granular soil applied contact and systemic insecticide and nematicide for the control of agricultural pests. It is a formulation of terbufos (S-*t*-butylthiomethyl-*O*,*O*-Diethyl phosphorodithioate) (Figure 1).

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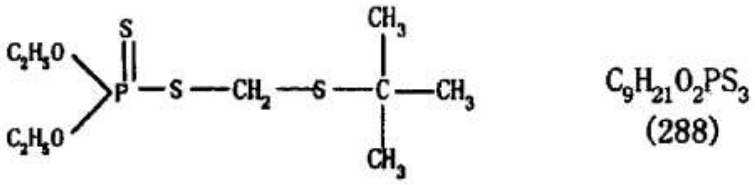

Figure 1. Molecular structure of terbufos, an organophosphate pesticide and active ingredient of the formulation Counter 15 FC. (Source: [24])

Terbufos is an organophosphate pesticide that inhibits cholinesterase by binding to the phosphate group on the enzyme [6-9]. Terbufos is classified as a type Ia pesticide by the World Health Organisation (WHO), and occupational exposure of farmers to it may result into death [10, 11]. Despite the frequent use of the formulation Counter 15FC by farmers, there is limited information of its effects on health. Few existing investigations focused on the active ingredient terbufos, and showed that male rats exposed to terbufos had a reduced fertility index when mated with non-exposed females [12]. Inhibition of cholinesterase enzymes by terbufos has been suggested [13]. Association between occupational use of terbufos and cancers (leukaemia, non-Hodgkin's lymphoma, prostate cancer and lung cancer) was also reported among pesticide applicators from Iowa and North Carolina in the USA [8].

In addition to terbufos, Counter 15FC contains 85% adjuvants with undisclosed characteristics. The adjuvants may potentiate toxicity of the pesticide formulation as previous studies have shown that some pesticides formulations are more toxic than their active ingredients [14]. Given that Counter 15FC is the form at which terbufos is supplied to farmers or end users, toxicological information on the formulation Counter 15FC will be more relevant to current exposure scenarios among humans. This study thus looked into the subchronic effects of the formulation Counter 15FC in male rats focusing on neurological, hepatic, renal, and reproductive function.

MATERIALS AND METHODS

Experimental animals

Twenty-five adult *albino Wistar* male rats weighing 167±16g bred in the animal house of the Medical Research and Applied Biochemistry Laboratory, Faculty of Health Science, University of Buea were used in the study. Breeding was done at standard laboratory conditions of 25°C with a 12 hours day light /12 hours darkness cycle. The animals were given feed and water *ad libitum*. The rats were acclimatized for one week before the start of the study. The protocol for this study was approved by the Institutional Animal Care and Use Committee review board of the University of Buea (UB-IACUC N^0 001/2019).

Reagents

Counter 15FC, a pesticide formulated and manufactured by Amvac Chemical Corporation (4100 E. Washington Blvd., Los Angeles, CA 90023), USA, and distributed in Cameroon by Cameroon Agrochem (Douala, Rue Joffre-Akwa, BP 5624 Douala), was purchased from Buea local market. Colorimetric kits for alanine aminotransferase (ALT) and aspartate aminotransferase (AST), creatinine and uric acid were obtained from Chronolab Systems, S.L. - Barcelona, Spain. A kit for determination of testosterone was purchased from Calbiotech Inc (Spring Valley, CA). S-Acetylthiocholine iodide (ACTI), S-Butyrylthiocholine iodide (BCTI), trichloroacetic acid, thiobarbituric acid, and 5,5'-dithio-bis -(2-nitrobenzoic acid) (DTNB) were purchased from VWR International S.A.S (France). The reagent 2, 4, 6-Tris (2-pyridyl)-S-Triazine) $_2$ (TPTZ) was obtained from Sigma Aldrich (Germany).

Experimental design

Twenty five male rats aged 2.5 months old were randomly divided into 5 groups of 5 animals each. Males were the focus in the current study rather than females, as previous investigations revealed altered fertility index in

male rats exposed to the active ingredient terbufos [12]. Group 1 (control) received corn oil used as a vehicle, while groups 2-5 were administered Counter 15FC through oral intubation at doses 0.1, 0.3, 1 and 3 mg/kg body weight (bwt), respectively (Table 1).

Table 1. Animal grouping with corresponding treatment

Group	Group 1	Group 2	Group 3	Group 4	Group 5
Number of animals	5	5	5	5	5
Corn oil (mL/kg bwt)	5	5	5	5	5
Counter 15FC (mg/kg bwt)	0	0.1	0.3	1	3

The lethal dose 50 (LD_{50}) for the active ingredient terbufos varies from 1.6 to 4.5 mg/Kg bwt in male rats when administered in corn oil [12]. Fractions of the LD_{50} were determined and used in the current subsonic study. Considering the highest LD_{50} value, 4.5 mg/kg bwt, the investigated doses corresponded to $LD_{50}/10$, $LD_{50}/30$, $LD_{50}/100$ and $LD_{50}/300$. The vehicle and pesticide were administered to rats 6 days per week for 9 weeks, with the animals weight recorded once a week. The animals were also observed for occurrence of signs and symptoms of toxicity including tremors and seizures. After the last dosing, the animals were fasted overnight, and their body weight recorded. The rats were euthanized with chloroform vapours, sacrificed by cervical dislocation and blood collected into dry tubes. Serum samples were obtained by blood centrifugation (181xg, 10 min, 4 ºC) and stored at -20 ºC for further biochemical analyses. Vital and reproductive organs (liver, kidneys, seminal vesicle, epididymis and testes) were dissected out and weighed. A 10% homogenate of each of the organs liver, seminal vesicle, epididymis and testes, was prepared in phosphate buffer (0.1M, pH7.4), centrifuged (181xg, 15 min, at 4^0C) and the supernatant stored at -20^oC for further biochemical assays.

Biochemical assessment of nervous, liver and kidney function

***Serum Cholinesterase activity*:** Cholinesterase activity was measured in serum samples using a method from Jonca et al. [15] with slight modifications, as reported previously [5]. Briefly, diluted serum sample (400 times; 50 µL/well) and DTNB (2 mM, prepared in 0.1 M phosphate buffer; pH 7.4, 50 µL/well) were mixed in a flat bottom 96-well plate and incubated for 30 minutes. A solution of ACTI (for AChE) or BCTI (for BuChE) was added and change in optical densities at 405 nm monitored for 15 minutes using a microplate reader. Cholinesterase activity (ChE) was determined as change in absorbance/minute/mL serum. Furthermore, the cholinesterase activities were expressed as percentage (%) variation compared to the control or vehicle group (%ChE) using the following formula:

$$\%\text{ChE} = \frac{\text{ChE} - \text{Average ChE for the control group}}{\text{Average ChE for the control group}} \text{x}100$$

Assessment of kidney and liver markers in serum: The level of creatinine and uric acid and activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed in serum samples using colorimetric kits from Chronolab, as reported elsewhere [16].

Testosterone and oxidative stress markers

Serum testosterone levels: Testosterone levels were quantified by ELISA competitive method, using a kit from Calbiotech, as per manufacturer's instructions. Briefly, 50 µL of either testosterone standard (0-18 ng/mL) or serum sample were pipetted into appropriate wells coated with streptavidin (in a 96 well plate). Testosterone- enzyme conjugate (100 µL) and anti-testosterone biotin reagent (50 µL) were added to all wells. The plate was incubated for 60 minutes, and all wells washed 3 times and blotted on absorbent paper. A 3, 3′, 5, 5′-Tetramethylbenzidine (TMB) solution was then added into the wells (100 µL /well), the plate incubated for 30 min and a stop solution added (50 µL/well). Absorbencies were recorded at 450 nm, and testosterone concentration in serum samples extrapolated from a standard curve.

Serum total antioxidant capacity (TAC): The TAC value was determined by the Ferric Reduction antioxidant

Power (FRAP) method [17]. Briefly, 5 µL Fe^{2+} standard solution (100 - 1000µM) or serum sample were pipetted into 96 well plates containing acetate buffer (300 mM, pH 3.6; 65 µL/well). A freshly prepared FRAP reagent (mixture of 25 mL of the acetate buffer, 2.5 mL of 10 mM TPTZ and 2.5 mL of 20 mM $FeCl_3.6H_2O$; 100 µL/well) was then added, absorbance read after 4 minutes at 595 nm, and FRAP value for each sample (expressed in µM Fe(II) equivalent) extrapolated from the standard curve.

Assessment of thiobarbituric acid reactive species, glutathione levels and catalase activity in organ homogenates: The homogenates were incubated with the substrate H_2O_2, a decreased absorbance of the mixture monitored at 240 nm from 30 - 90 seconds, and catalase activity (CAT) expressed as pmole H_2O_2/min using the substrate molar extinction coefficient (39.4×10^3 $M^{-1}cm^{-1}$) [18]. For assessment of 2-thiobarbituric acid reactive substances (TBARS), the reaction mixture containing sample homogenate, 2-thiobarbituric acid and 20 % trichloracetic acid, was incubated at 90 °C for 1 hour. The mixture was cooled, centrifuged the absorbance of the supernatant measured at 540 nm and TBARS levels calculated from the molar extinction coefficient (1.56×10^5 cm^2/mmole) [19]. Each organ's homogenate was incubated with Ellman's reagent (DTNB solution) for 1 hour, the absorbance measured at 412 nm and glutathione levels (GSH) calculated using the molar extinction coefficient 13600 $M^{-1}cm^{-1}$ [20] . The latter biochemical parameters were corrected using protein levels in the organ homogenates, which were determined according to Gornall et al. [21].

Statistical analysis

All values were expressed as the mean ± SD. Data were assessed for normality using Kolmogorov-Smirnov test, and statistical significance between groups evaluated by one-way ANOVA. Pairwise comparison was done using Student Newman-Keuls test, and Pearson's correlation coefficients determined for assessment of a relationship between exposure levels and investigated parameters. Values of $P < 0.05$ were considered as significant. Data were analysed using MedCalc v14.8.1.0 software.

RESULTS

Effects of counter 15 FC on animal body and organ weights, neurotoxicological signs and lethality

After exposure of the rats to Counter 15FC for 9 weeks, there was no change in body weight gain nor sign of toxicity for the groups treated with pesticide doses below 3 mg/kg bwt. However, exposure to the highest investigated pesticide dose 3 mg/kg bwt resulted into tremors/ seizures and lethality. Four rats from the latter group died between days 3 - 6, and the remaining one animal showing exacerbation of the toxicological signs was euthanized on day 7 (Table 2).

Table 2. Number or percentage of animal's dead or presenting tremors/seizures in different groups after six days of experiment

Dose of Counter 15FC (mg/kg bwt)	Symptoms (x/N)	Mortality (x/N)
0	0/5 (0%)	0/5 (0%)
0.1	0/5 (0%)	0/5 (0%)
0.3	0/5 (0%)	0/5 (0%)
1	0/5 (0%)	0/5 (0%)
3	5/5 (100%)	4/5 (80%)

Investigate symptoms include seizures and tremors. N= Total number of animals in the group rats at the beginning of the experiment; n= number of animals dead or presenting seizures/tremors. Values in brackets represent percentage of animal's dead or presenting seizures/tremors.

The body weight gain of rats treated with either pesticide (0.1, 0.3, 1 mg/kg bwt) or vehicle (corn oil) was assessed during 9 weeks. As shown in Figure 2, the pesticide formulation at the dose of 1 mg/kg bwt or less, did not altered the animal body weight significantly when compared to the vehicle group. Likewise, the relative (Figure 3) and absolute (data not shown) weight of the organs investigated were not altered by pesticide exposure.

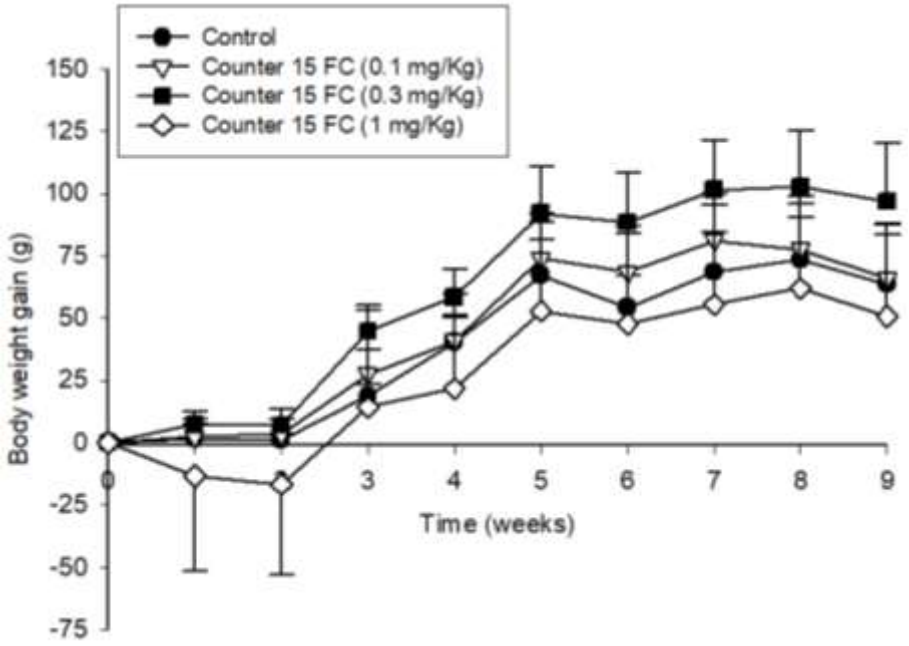

Figure 2.Change in body weight gain of rats during 9 weeks exposure to corn oil (vehicle) or Counter 15 FC.
Body weight gain for each animal was determined using the formula B_i-B_0, where B_0 and B_i represent the initial (at week 0) and final (at week i, with 0≥i≥9) body weight of the animal, respectively. Each data point represents the mean ± SD of 5 animals.

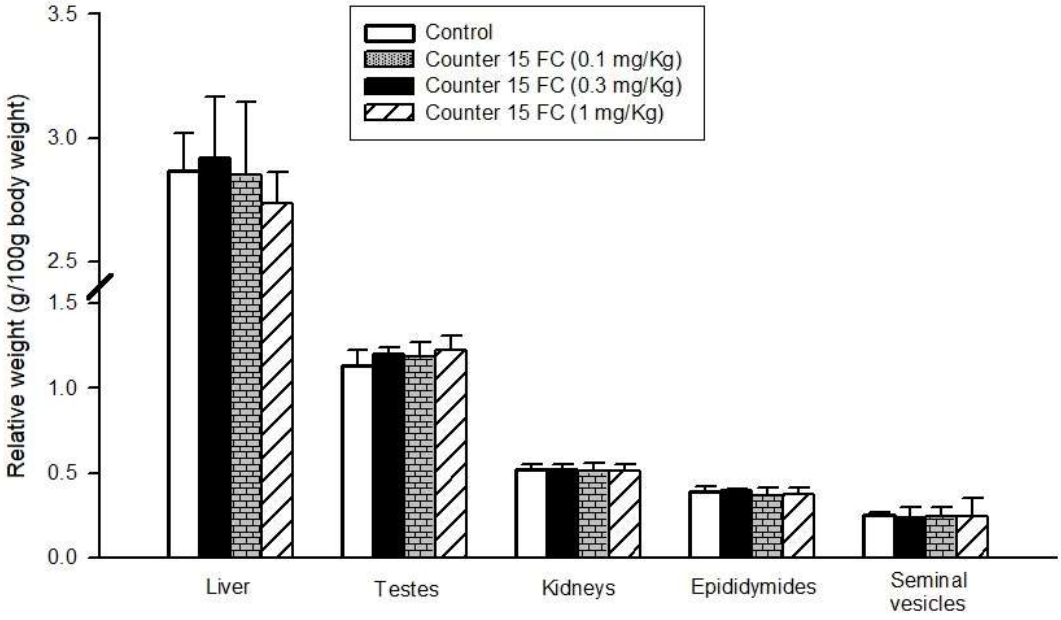

Figure 3. Relative organ weight of rats after 9 weeks of treatment with vehicle (corn oil) or Counter 15FC.
Relative weight of each organ was determined using the formula (b/B) x100%, where b and B represent the organ weight and animal body weight, respectively). Each data point represents the mean ± SD of 5 animals.

Effects of counter 15FC on serum cholinesterase activities

As shown in Figure 4, exposure to the pesticide formulation at the dose 1 mg/kg bwt resulted in significant inhibition of serum AChE and BuChE activities in rats after 9 weeks. Moreover, cholinesterase inhibition negatively correlated with the pesticide dose (r=-0.96 and P=0.039 for AChE; r=-0.98 and P=0.023 for BuChE).

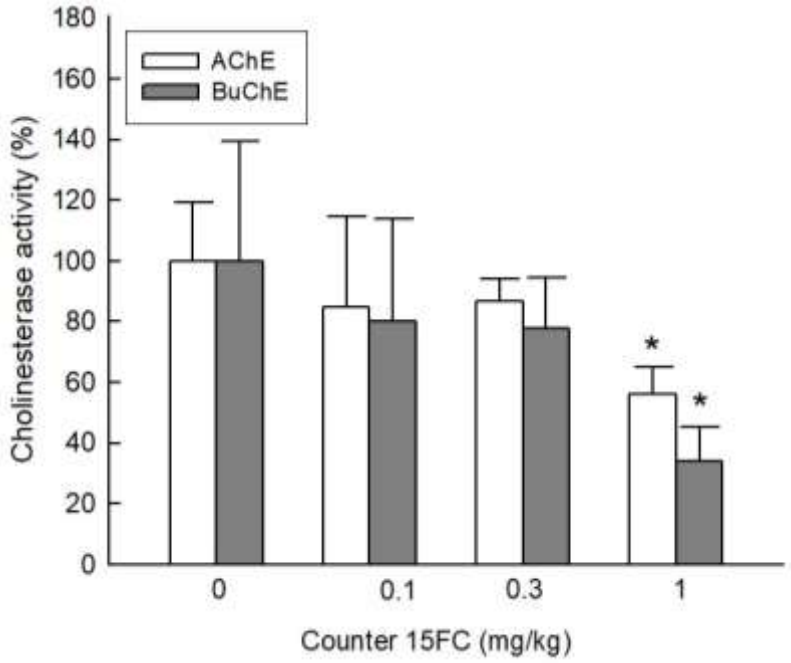

Figure 4. Variation of acetylcholinesterase and butyrylcholinesterase activities relative to the control group in serum of rats treated with corn oil or Counter 15FC for 9 weeks.

Values are expressed in percentage relative to the control group. Significant difference at P<0.05 (Student Newman Keuls test) when compared to the corresponding control. AChE= acetylcholinesterase, BuChE= butyrylcholinesterase

Effects of Counter 15FC on serum biomarkers of liver and kidney function

Liver and kidney functions were assessed through serum activities of aminotransferase enzymes and concentrations of creatinine and uric acid, and results presented in Table 3.

Table 3. Serum biochemical markers for liver and kidney function in rats after exposure to corn oil and counter 15FC for 9 weeks.

Counter 15FC (mg/kg bwt)	ALT (U/L)	AST (U/L)	Creatinine (µM)	Uric acid (µM)
0	26.66 ± 18.36	94.38 ± 17.02	50.51 ± 7.22	226.42 ± 95.73
0.1	28.18 ± 3.83	103.83 ± 37.29	63.50 ± 13.64	248.95 ± 36.79
0.3	28.35 ± 6.96	96.54 ± 19.78	51.24 ± 17.93	197.40 ± 67.89
1	30.92 ± 6.75	84.70 ± 19.95	48.35 ± 11.58	200.84 ± 41.79

ALT= alanine aminotransferase activity, AST= aspartate aminotransferase activity. Each data point represents the mean ± SD of 5 animals. No significant difference at P<0.05 (Student Newman Keuls test)

Creatinine and uric acid levels did not differ significantly between the pesticide -exposed groups and control animals. Likewise, ALT and AST activities in rats exposed to Counter 15FC did not vary significantly when compared to the vehicle group. However, further correlation analysis revealed a significant and positive association between ALT and pesticide dose (r=-0.96 and P=0.036).

Effects of Counter 15FC on serum testosterone levels

Testosterone levels were assessed in serum of the animals exposed to the pesticide formulation for 9 weeks (Figure 5). All experimental groups exposed to Counter 15FC did not show any alteration of the hormone levels in male rats when compared to the vehicle group.

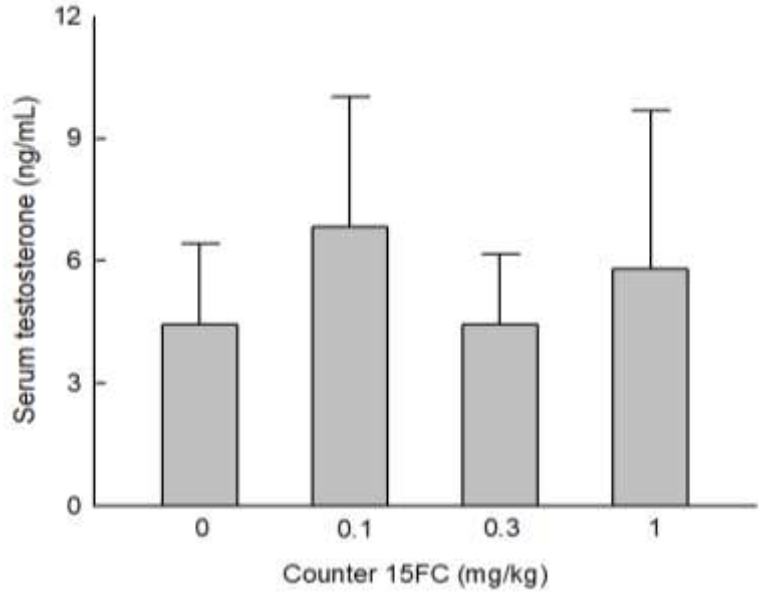

Figure 5. Serum testosterone levels in rats treated with Counter 15FC for 9 weeks. No significant difference between groups (Student Newman Keuls test)

Oxidative stress biomarkers in the animals following exposure to counter 15FC

Oxidative stress was assessed in the experimental animals through TBARS, GSH and CAT in homogenates from liver, testis, epididymis and seminal vesicles (Table 4), as well as TAC in serum (Figure 6). Testicular CAT was inhibited ($P<0.05$) in rats exposed to 0.1 and 0.3 mg counter 15FC / kg bwt, while other parameters remained not affected significantly.

Table 4. Levels of TBARS, glutathione and activity of catalase in liver, testis, epididymis and seminal vesicles in rats exposed to corn oil and/or Counter 15FC for 9 weeks.

Organ	Counter 15FC (mg/kg bwt)	TBARS (nmole/mg protein)	GSH (µmole/mg protein)	Catalase (pmole H_2O_2/min/mg protein)
Liver	0	1.20 ± 0.38	57.81 ± 18.52	87.27 ± 42.09
	0.1	1.26 ± 0.50	44.15 ± 10.50	148.79 ± 145.46
	0.3	1.16 ± 0.23	49.43 ± 7.04	152.35 ± 97.29
	1	1.37 ± 0.09	37.52 ± 9.21	207.54 ± 160.13
Testis	0	0.24 ± 0.01	7.05 ± 2.00	32.47 ± 11.81
	0.1	0.17 ± 0.07	7.52 ± 1.11	12.69 ± 3.65*
	0.3	0.20 ± 0.08	7.05 ± 1.23	12.20 ± 2.89*
	1	0.22 ± 0.07	8.09 ± 1.64	25.36 ± 8.84
Epididymis	0	52.27 ± 10.94	1.64 ± 0.24	1.99 ± 0.56
	0.1	45.33 ± 12.43	1.55 ± 0.39	2.95 ± 0.76
	0.3	44.28 ± 12.54	1.55 ± 0.24	1.90 ± 0.33
	1	56.78 ±18.43	2.09 ± 0.47	2.25 ± 0.59
Seminal vesicles	0	168.89 ± 45.08	3.17 ± 0.71	10.50 ± 1.81
	0.1	134.75 ± 16.33	2.21 ± 0.67	8.44 ± 2.10
	0.3	122.85 ± 21.11	2.98 ± 1.04	6.81 ± 1.04
	1	101.22 ± 18.05	2.42 ± 0.84	12.27 ± 1.10

TBARS= Thiobarbituric acid reactive substances; GSH= Glutathione. Significant difference when compared to the respective control group at *P<0.025 (Student-Newman-Keuls test)

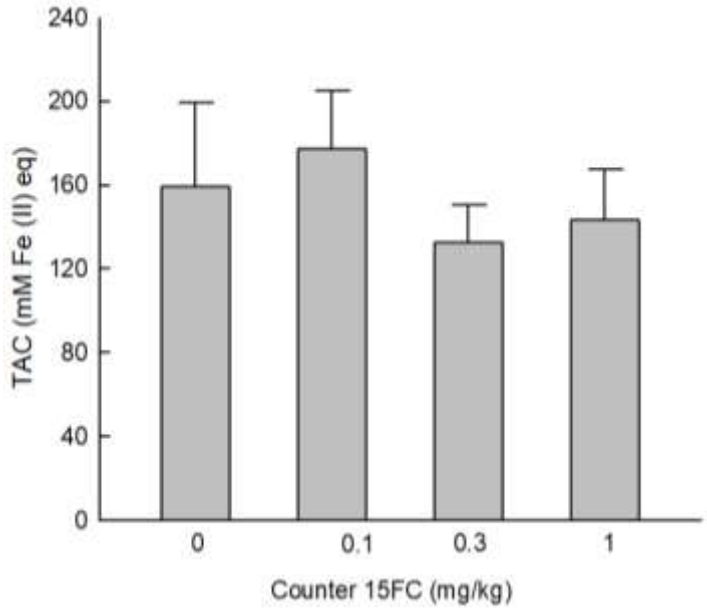

Figure 6. Serum total antioxidant capacity in rats treated with a vehicle (corn oil) or Counter for 9 weeks. No significant difference between groups (P>0.05)

DISCUSSION

This study was designed to investigate the subchronic effects of Counter 15FC formulation on liver, kidney, reproductive and neurological functions in adult male rats. At 3 mg/kg bwt which was the highest dose investigated, the pesticide formulation induced adverse changes in the animals' neurological function as from the second day of exposure with the symptoms including tremor and seizures. These results are similar to those reported by Weiner et al. [22] after acute oral exposure of rats to pyrethroid insecticides. The latter dose of Counter 15FC was also lethal to 4 rats within the first week of experiment. This lethality at a relatively low dose of the pesticide formulation, 3 mg/kg bwt (corresponding to 0.45 mg terbufos/kg bwt), corroborates classification of terbufos as type Ia (extremely hazardous) pesticide, as well as reported fatal poisoning in a 43-year-old farmer following occupational exposure to the pesticide active ingredient [10,11]. Tremors reflect persistent activation of muscarinic acetylcholine receptors [23], and the observed symptoms could be related to neurological dysfunction. The other experimental animals exposed to Counter 15FC at doses ≤1mg/kg bwt could survive toxicity of the pesticide formulation for 9 weeks, and were sacrificed for assessment of biochemical parameters including cholinesterase activities.

Both AChE and BuChE activities decreased in the animals exposed to Counter 15FC at the dose 1 mg/kg bwt. The decrease in these enzyme activities may be related to the phosphorothionate terbufos, which is the active ingredient of Counter 15FC [5, 8, 13]. The AChE activity also correlated with increased pesticide dose, suggesting that altered behavioural effects and lethality occurring at the higher dose of Counter 15FC, 3 mg/kg bwt, resulted from drastic cholinesterase inhibition in the animals. In fact, AChE main function consists in hydrolysis of acetylcholine into acetate and choline at cholinergic synapses [26]. AChE is present in several tissues including nervous tissues and blood [26-28], and alteration in its activity may reflect the status of the nervous system. Upon exposure of the animals to Counter 15FC, terbufos is activated to its neurotoxic form via oxidative desulfuration, one of the most important cytochrome P450-mediated reactions involving organophosphates. The active metabolite thereafter inhibits AChE by binding to the phosphate group on the enzyme. Following AChE inhibition, free acetylcholine accumulates at the nerve endings of all cholinergic nerves

and causes overstimulation of electrical activity [6, 9, 24]. The enzyme BuChE, also known as pseudo-cholinesterase, has been suggested to be implicated in regulation of acetylcholine levels in the central nervous system [29]. Therefore, it could be speculated that exacerbation of seizure or tremors in the animals exposed to 3 mg/kg bwt Counter 15FC also resulted from inhibition of BuChE enzyme, which acts as a backup for AChE and scavenger for poisons inhibiting AChE activity [25].

Decreased AChE activity was reported in male farmers from Buea in Cameroon, who were using agro pesticides including the terbufos based formulation Counter 15FC [5]. Also, Kim et al. [30] reported inhibited brain and plasma cholinesterase and subsequent death in rats exposed to the active ingredient terbufos at the dose 0.5 mg/kg bwt for 2 days. It is worth noting that the dose 0.5 mg/kg bwt is closer to 0.45 mg terbufos/kg bwt in the Counter 15FC formulation, which was lethal to the animals as from the third day of the current study. However, the terbufos-based formulation, Counter 15FC, did not alter body weight and weight of selected organs including liver, testis, epididymis and seminal vesicles of animals exposed to Counter 15FC at the doses ≤1mg/kg bwt. The latter organs were further assessed through oxidative stress biomarkers including TBARS, GSH and CAT.

Catalase activity was significantly inhibited in testes from the animals exposed to Counter 15FC at doses 0.1 and 0.3 mg/kg bwt. Reduction in catalase activity was also reported in testes of rats exposed to the pesticide Parastar (a formulation containing lambda-cyhalothrin and imidacloprid as active principles) at the dose 2.49 mg/kg bwt [4]. Moreover, Lu et al. [31] reported significant reduction of catalase expression by the pesticide chlorpyrifos. Catalase inhibition suggests alteration of the testicular antioxidant system, which may result into oxidative stress, though the lipid peroxidation index TBARS, and GSH which is substrate of the important antioxidant enzyme superoxide dismutase, were not affected. Catalase catalyses decomposition of the reactive oxygen species (ROS) hydrogen peroxide into water and oxygen. Catalase is a very important antioxidant enzyme in reducing ROS levels thereby preventing damage of tissues/cells by the ROS [32]. Therefore, inhibition of testicular catalase may result into oxidative damage of testicular cells and impairment in spermatogenesis, leading to infertility in the male rats [33]. Increased ROS production was reported earlier in mouse immortalized spermatogonia exposed to the active principle terbufos. Terbufos induced apoptosis and DNA damage in the testicular cell lines [34] and should be considered potentially hazardous to testis. Exposure to terbufos also resulted into altered fertility index in male rats [11]. It should be however noted that inhibition of CAT activity in the current study displayed a nonmonotonic dose-response pattern. Such observation was reported earlier following subchronic exposure of male rats to a pesticide formulation Parastar, at a dose 2.49 mg/kg bwt. Several studies have also reported nonmonotonic dose-response adverse effects with pesticides and other endocrine disrupting chemicals, as reviewed elsewhere [35]. The testicular function was further explored through assessment of serum testosterone levels, which is a steroid male hormone mainly synthesized in the testis.

The testosterone levels were not altered by sub-chronic exposure to Counter 15FC. Similarly, inhibition of testicular catalase without significant alteration on serum testosterone levels was reported in rats exposed to the pesticide formulation Parastar at the dose 2.49 mg/kg bwt [4]. As testicular TBARS levels were not affected, it could be speculated that testicular oxidative stress was still moderate, and the steroidogenic function preserved. However, a possibility of endocrine disruption may not be totally rolled out, and may be further explored through assessment of other reproductive hormones such as gonadotrophins.

Liver tissues have been confirmed to be the primary target organ for organophosphate pesticides [36]. The organophosphates cause possible changes in serum biomarker levels for liver damage, AST and ALT. Nevertheless, these biochemical markers were not altered in rats following sub-chronic exposure to the formulation Counter 15FC, which contains the organophosphate terbufos. Likewise, there was no significant change in liver weight in the animals exposed to the pesticide formulation, suggesting integrity of the liver organ. This was further emphasised by non-significant changes in liver oxidative stress biomarkers GSH, CAT and TBARS.

Although there was no significant difference in liver GSH level and serum ALT activity between groups, further analysis of data revealed a 35% decrease in GSH compared to the control group, as well as a positive correlation between ALT and pesticide exposure levels. As ALT is a cytosolic enzyme mainly expressed by liver cells, the latter correlation implies that increment of pesticide dose exacerbates hepatocytes lysis (hepatotoxicity) in the animals. Such increase in ALT has been reported among farmers from Buea who were using agro pesticides [16]. GSH is involved in prevention of lipid peroxidation in cells by scavenging for free radicals [33, 37]. Therefore, a possibility of GSH depletion following subchronic exposure to Counter 15FC at concentrations >1 mg/kg bwt, or chronic exposure may not be totally ruled out. Pearson and Patel [38] stated that most acute and sub-acute exposures to organophosphate compounds decreased GSH level.

Another key organ in elimination of xenobiotic is the kidney. Creatinine and uric acid serve as biochemical markers for kidney damage, and their increase/accumulation in serum is interpreted as failure of the kidneys to eliminate these compounds. Results from the current study did not reveal significant change in these biomarkers following exposure to counter 15FC, implying that the pesticide formulation did not alter kidney function when administered to the animals at ≤1mg/kg bwt for 9 weeks. As kidney damage in animals exposed to some organophosphorus compounds such as diazinon, chlorpyrifos, malathion and methyl parathion has been reported [39-41], the current report warrants further investigations, preferably combination of several functions and damage markers, which has been shown to improve diagnosis of kidney failure [42].

CONCLUSIONS

Exposure to a terbufos based pesticide, Counter 15FC at relatively lower doses resulted in lethality, altered neurologic system and testicular oxidative stress in male rats. Therefore, the pesticide formulation should be handled with proper measures in order to mitigate its adverse effects among farmers.

Authors' contributions:

DZC performed experiments, collected data and proposed the first draft; FPTM conceived the experiment, constructed figures and improved the manuscript; EAN, DZ and JCGA provided substantial contribution related to conception of the work and improved the manuscript.

Conflict of interest disclosure

There is no conflict of interest.

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