

Inductions of CaC₂ on Sperm Morphology and Viability of the Albino Mice (*Mus musculus*)

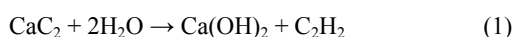
Dike H. Ogbuagu, Etsede J. Oritsematosan

Abstract—This work investigated possible inductions of CaC₂, often misused by fruit vendors to stimulate artificial ripening, on mammalian sperm morphology and viability. Thirty isogenic strains of male albino mice, *Mus musculus* (age ≈ 8 weeks; weight = 32.5 ± 2.0 g) were acclimatized (ambient temperature 28.0 ± 1.0 °C) for 2 weeks and fed standard growers mash and water *ad libitum*. They were later exposed to graded toxicant concentrations (w/w) of 2.5000, 1.2500, 0.6250, and 0.3125% in 4 cages. A control cage was also established. After 5 weeks, 3 animals from each cage were sacrificed by cervical dislocation and the cauda epididymis excised. Sperm morphology and viability were determined by microscopic procedures. The ANOVA, means plots, Student's t-test and variation plots were used to analyze data. The common abnormalities observed included Double Head, Pin Head, Knobbed Head, No Tail and With Hook. The higher toxicant concentrations induced significantly lower body weights [$F_{(829,899)} > F_{crit(4,19)}$] and more abnormalities [$F_{(26,52)} > F_{crit(4,00)}$] at $P < 0.05$. Sperm cells in the control setup were significantly more viable than those in the 0.625% ($t = 0.005$) and 2.500% toxicant doses ($t = 0.018$) at the 95% confidence limit. CaC₂ appeared to induced morphological abnormalities and reduced viability in sperm cells of *M. musculus*.

Keywords—Artificial ripening, Calcium carbide, fruit vendors, sperm morphology, sperm viability.

I. INTRODUCTION

CALCIUM CARBIDE (CaC₂) in its pure state is colourless, though most of the industrially produced impure ones are black or grayish-white in colour, depending on the quality. The main use of CaC₂ is in the production of flammable acetylene gas for welding and fabrication works. According to [1], once in contact with moisture, CaC₂ releases acetylene, a gas which also has fruit ripening characteristics, similar to ethylene. The reaction is as:



In natural conditions, fruits get ripened by the action of a ripening hormone in the fruit. During this process, a wide spectrum of biochemical changes takes place, such as chlorophyll degradation, biosynthesis of carotenoids (antioxidants, immune system booster, anti-cancer agent), anthocyanins (powerful antioxidants), essential oils, as well as flavour and aroma components [2]. A similar action is also

induced by the action of CaC₂ in artificial conditions. So, this has created a trend of the use of carbide gas from CaC₂ to ripen fruits in especially our cities; a practice that could portend hazard to the human body, especially as the chemical could also contain traces of arsenic and phosphorus [2]. Direct consumption of acetylene has been found to be detrimental as it reduces oxygen supply to the brain and can further lead to prolonged hypoxia [3]. Impurities like arsenic and phosphorus found in industrial grade CaC₂ may cause dizziness, frequent thirst, irritation in mouth and nose, weakness, permanent skin damage, difficulty in swallowing, vomiting, and skin ulcer among workers who are in direct contact with these chemicals while applying the ripening agent. Higher exposures may even cause undesired fluid build-up in lungs (pulmonary edema) [4]. According to [5], chemicals including CaC₂ and acetylene gas have also shown other adverse effects including memory loss, neurological system failure, cerebral edema, colonic lung cancer, quick-buck syndrome [6], deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and hematological changes [7], and also proliferation of bacteria, fungus and viruses which can cause diarrhea, peptic ulcer and other human diseases when used to ripen fruit.

Although the practice of ripening fruits with carbide has been banned in many countries [7], it is still freely used in Nigeria. Fruit farmers, vendors and wholesalers in some cities of the country rampantly use carbide gas to ripen a variety of fruits such as bananas, mangoes, pineapples, and papayas in commercial centres and market places.

The aim of this research was to ascertain possible qualitative and quantitative inductions of CaC₂ on the morphology and viability of sperm cells of the albino mice, *Mus musculus*. The objectives included determination of possible inductions of CaC₂ on sperm cell morphology of the mice, enumeration of such morphological inductions, as well as determination of possible effects on sperm cells viability.

II. METHODOLOGY

A. Acquisition and Acclimatization of Test Organisms

A total of 30 isogenic strain of male albino mice (*M. musculus*), about 8 weeks old, weighing 32.5 ± 2.0 g, and purchased from the Imo State Polytechnic Animal House, Nigeria on the 26th of June 2014, were used as experimental model. The animals were randomly divided into five groups and placed in 530x350x230 cm cages with perforated roof lids. The standard cages were bedded with dry wood shavings which were changed every day to prevent growth of maggots. They were acclimatized in the Pollution Laboratory of the Federal University of Technology, Owerri, Nigeria at average

D. H. Ogbuagu is with the Department of Environmental Technology, Federal University of Technology, PMB 1526, Owerri, Nigeria (corresponding author, phone: 234-7038850065; e-mail: dike.ogbuagu@futo.edu.ng).

J. O. Etsede is a research project student with the Department of Environmental Technology, Federal University of Technology, Owerri, Nigeria (e-mail: justiceetsede@gmail.com).

ambient temperature of $28.0 \pm 1.0^\circ\text{C}$ for 2 weeks. During this period, they were fed about 20g standard rodent growers mash (produced by Bendel Food and Flour Mills Nigeria Ltd) and water *ad libitum*. Dead animals were watched out for and removed as soon as possible to avoid contamination.

B. Formulation of Toxicant

CaC_2 was obtained from a welding and fabrications workshop in Obinze, Owerri, Nigeria and ground to powder in a dry mortar. Four different concentrations of the toxicant, viz, 0.5g, 0.25g, 0.125g and 0.0625g were weighed out and incorporated to make up 20g of toxicant-feed mixture each. This translates to 2.5000%, 1.2500%, 0.6250% and 0.3125% toxicant concentrations in 20g feeds (w/w) respectively.

C. Exposure of Test Organisms

After 2 weeks of acclimatization, 5 mice were distributed in five different cages labeled C1, C2, C3, C4 and CC in descending order of intended toxicant concentrations, with CC as the control cage. Thereafter, the animals were fed the different toxicant concentrations i.e. 0.5g (2.5000%), 0.25g (1.2500%), 0.125g (0.6250%) and 0.0625g (0.3125%) in 20g toxicant-feed mixture. They were also given water *ad libitum*.

D. Animal Sacrifice and Semen Collection

After exposure period of 5 weeks (35 days), i.e. the peak of spermatogenesis [8], 3 randomly selected mice from each treatment and control cage were weighed. The animals were sacrificed by cervical dislocation and quickly dissected to remove the testes and epididymis. The caudal epididymis was excised and placed in Petri-dishes containing 1ml of physiological saline. They were then minced and teased carefully with fine scissors and forceps to release the spermatozoa.

E. Sperm Viability test

In order to estimate the percentage viability of the sperm cells after excision, a drop of semen so collected was placed on a pre-cleaned, grease-free glass slide with a dropper and covered with a slip. Observation under the X10 magnification of a light microscope was made for progressive motility and X40 objective to count the number of viable and non-viable spermatozoa. The viable spermatozoa remained unstained while the non-viable ones stained red.

F. Sperm Morphology Test

After gentle pipetting of the released spermatozoa to separate the suspension from the tissue fragment, a drop of 1% Eosin Y solution (10:1) was added and allowed for 30 minutes. A clean glass slide was angularly positioned at 30° to spread a drop of the sperm suspension through the whole length of another clean, grease-free glass slide. The smears were air-dried, coded, and examined under X40 magnification of a light microscope. Three separate slides were prepared for each group (C1-CC). Observation and scoring was done in up to 15 fields of view using the tally counter. Each mouse was assessed for 800 sperm cells for morphological aberration according to the criteria of [8] and [9].

G. Statistical Analysis

Bivariate and multivariate analyses as provided by the SPSS v.22 and MS Excel v.2010 softwares were used to analyze data. Variation plot was used to represent number of morphological abnormalities in sperm cells. The single factor ANOVA was used to determine homogeneity in mean variance of weights and abnormalities induced by the different concentrations of the toxicant at $P < 0.05$. Means plots were used to detect structure of group means, and the student's t-test was used to make pair-wise comparison in sperm viability inductions at the 95% confidence limit.

III. RESULTS

A. Effect of Toxicant on Weight of Organisms

Table I shows the weight of the animals in different concentrations of the toxicant after 5 week exposure period. The highest mean weight ($42.59 \pm 2.97\text{g}$) of animals was recorded in the 0.6250% (0.125g of toxicant in 20g of feed) concentration, while the least mean weight of $34.97 \pm 1.82\text{g}$ was recorded in the 0.3125% (0.0625g of toxicant in 20g of feed) concentration. Animals exposed to the 2.5000% (0.5g toxicant in 20g of feed) recorded mean weight of $40.61 \pm 3.87\text{g}$, while the mean weight of $39.40 \pm 0.11\text{g}$ was recorded in the 1.2500% (0.25g of toxicant in 20g of feed) concentration. However, animals in the control experimental setup recorded mean weight of $39.60 \pm 1.08\text{g}$.

The ANOVA test revealed that the different concentrations of toxicant induced significantly different weights of the organisms [$F_{(829.899)} > F_{\text{crit}(4.19)}$] at $P < 0.05$.

TABLE I
WEIGHTS OF MICE (*M. MUSCULUS*) EXPOSED TO DIFFERENT
CONCENTRATIONS OF CaC_2 AFTER 5 WEEKS

Concentrations/ 20g of feed (%)	Weight (g)			Mean weight \pm SE
	Replicate 1	Replicate 2	Replicate 3	
0.000g (0.00%)	38.93	41.72	38.16	39.60 ± 1.08
0.0625g (0.3125%)	38.60	32.96	33.34	34.97 ± 1.82
0.125g (0.6250%)	40.54	38.78	48.45	42.59 ± 2.97
0.25g (1.2500%)	39.56	39.20	39.45	39.40 ± 0.11
0.5g (2.5000%)	42.65	33.13	46.05	40.61 ± 3.87

B. Morphological Abnormalities in Sperm Cells

The common abnormalities observed in sperm cells of the mice included Double Head (DH), Pin Head (PH), Knobbed Head (KH), No Tail (NT), and With Hook (WH). The most abnormalities (1.09 ± 0.15) were recorded in the 1.2500% toxicant concentration, while the least abnormalities (0.50 ± 0.38) were observed in the control animals (Table II). In all, 0.86% of sperm cells enumerated in this study were abnormal. The order of abnormalities induction by toxicant concentrations is $1.2500\% (1.09 \pm 0.15) > 2.500\% (1.05 \pm 0.36) > 0.6250\% (0.84 \pm 0.21) / 0.3125\% (0.84 \pm 0.29) > 0.0000\% (0.50 \pm 0.38)$.

Cumulative mean sperm cell abnormalities in the various toxicant concentrations are shown in Fig. 1.

The ANOVA test revealed that the different toxicant concentrations induced significantly different quantitative

abnormalities in the sperm cells examined [$F_{(26,52)} > F_{crit(4,00)}$] at $P < 0.05$. The post-hoc structure of group means that utilized the different concentrations of the toxicant as predictor variable revealed that the 0.3125% toxicant concentration mostly induced the DH abnormality (Fig. 2), the 0.625% and 1.25% toxicant concentrations mostly induced PH abnormality

(Fig. 3), the 0.3125% toxicant concentration mostly induced KH abnormality (Fig. 4), the 1.25% and 2.50% toxicant concentrations mostly induced NT abnormality (Fig. 5), while the control and 2.50% toxicant concentrations mostly induced WH abnormality (Fig. 6).

TABLE II
MORPHOLOGICAL ABNORMALITIES (MEAN \pm SE) IN SPERM CELLS INDUCED BY DIFFERENT CONCENTRATIONS OF CAC_2 IN MICE (*M. MUSCULUS*) AFTER 5 WEEKS OF EXPOSURE

% toxicant concentration/ 20 g of feed	Abnormalities					Total number of abnormal sperms	% abnormal sperms
	DH	PH	KH	NT	WH		
0.0000%(CC)	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	4.00 \pm 3.06	4.00 \pm 3.06	0.50 \pm 0.38
0.3125%(C4)	0.67 \pm 0.67	1.00 \pm 1.00	2.33 \pm 1.45	1.33 \pm 1.33	1.33 \pm .67	6.67 \pm 2.33	0.84 \pm 0.29
0.6250%(C3)	0.00 \pm 0.00	2.00 \pm .00	2.00 \pm 0.00	1.00 \pm 1.00	1.67 \pm .67	6.67 \pm 1.67	0.84 \pm 0.21
1.2500%(C2)	0.00 \pm 0.00	0.00 \pm 0.00	5.00 \pm 0.56	1.67 \pm 0.33	2.00 \pm 0.58	8.67 \pm 1.20	1.09 \pm 0.15
2.5000%(C1)	0.00 \pm 0.00	0.67 \pm 0.67	2.00 \pm 2.00	2.00 \pm 1.00	3.67 \pm 1.20	8.33 \pm 2.91	1.05 \pm 0.36
Total No of cells	2	11	34	18	38	103	0.86%

Total number of sperm cells scored=12000, Number of sperm cells scored at each toxicant concentration=800, DH = Double Head, PH= Pin Head, DT=Double Tail, KH=Knobbed Head, NT=No Tail, WT=With Hook

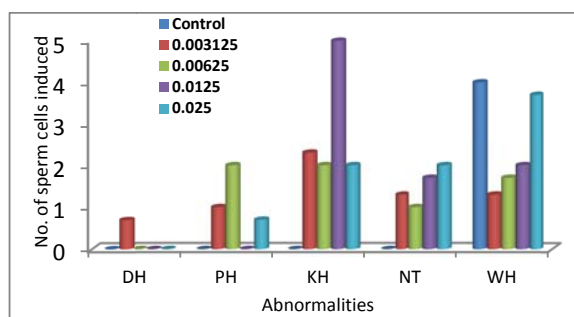


Fig. 1 Mean morphological abnormalities in sperm cells of albino mice (*M. musculus*) induced by different concentrations of CaC_2 after 5 weeks

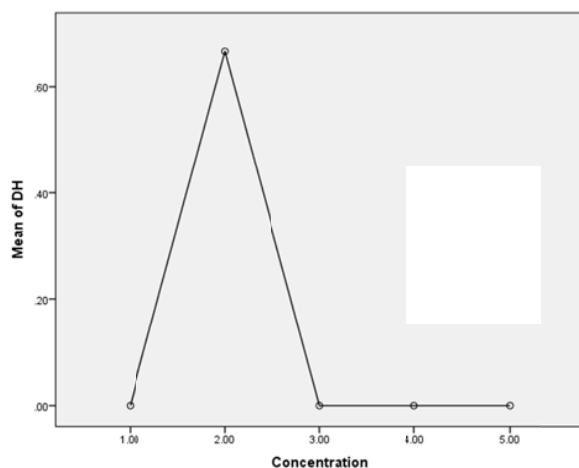


Fig. 2 Means plot in Double Head (DH) sperm abnormality by toxicant concentrations

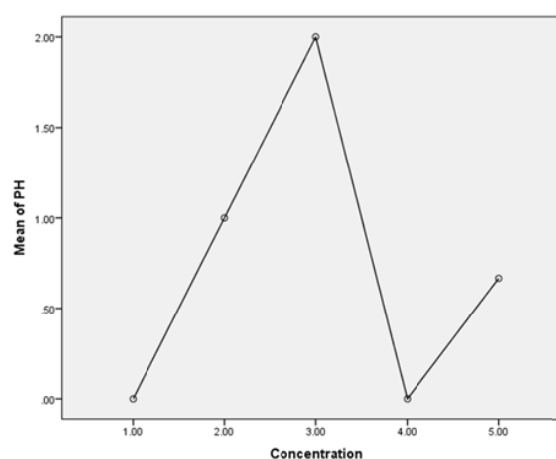


Fig. 3 Means plot in Pin Head (PH) sperm abnormality by toxicant concentrations

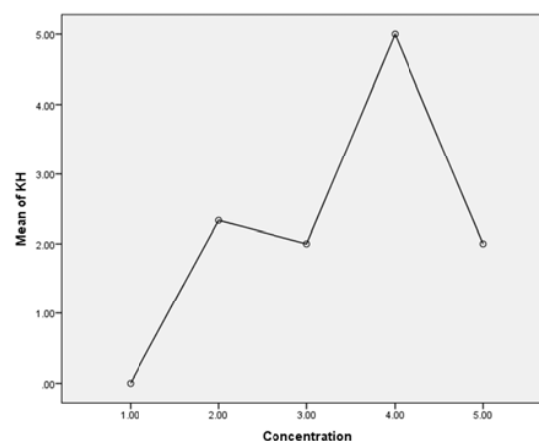


Fig. 4 Means plot in Knobbed Hook (KH) sperm abnormality by toxicant concentrations

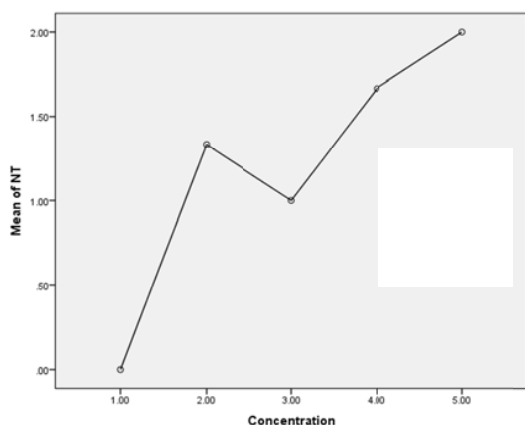


Fig. 5 Means plot in No Tail (NT) sperm abnormality by toxicant concentrations

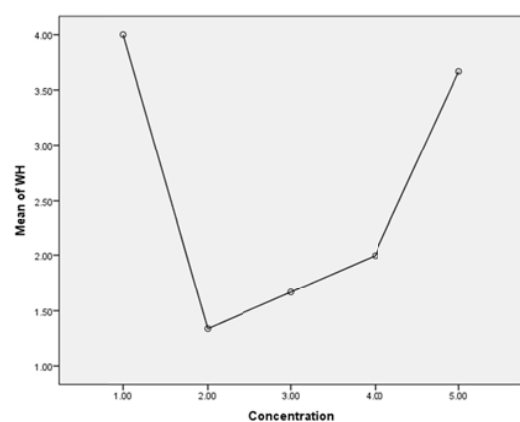


Fig. 6 Means plot in With Hook (WH) sperm abnormality by toxicant concentrations

C. Viability of Sperm Cells

Sperm cells in the control experiment were about 92% viable, while those in the 0.3125% toxicant concentration were 78% viable. However, sperm cells in the 0.6250%, 1.2500% and 2.5000% toxicant concentrations were 71.7, 62.0 and 60.0% viable respectively (Table III). In numerical terms, 728, 760 and 720 sperm cells were viable in the respective replicates of the control setup, while 544, 640 and 688 sperm cells were viable in the 0.3125% toxicant concentration replicates. In the 1.2500% toxicant concentration, 480, 496 and 512 sperm cells were viable in the replicates, while in the 2.5000% toxicant concentration, 480, 440 and 520 sperm cells were viable in the respective replicate concentrations.

The number of viable sperm cells in the organisms exposed to the different toxicant concentrations differed significantly [$F_{(482,78)} > F_{crit(4,19)}$] at $P < 0.05$. The student's t-test revealed that the cells were significantly more viable in the control mice (CC) than in all of those exposed to the 0.625% toxicant concentration (C3) ($t=0.005$), 1.250% toxicant concentration (C2) ($t=0.014$), and 2.5000% toxicant concentration (C1) ($t=0.018$) at the 95% confidence level. Additionally, the number of viable sperm cells in the 0.6250% toxicant

concentration (C3) also differed significantly from that in the 1.2500% (C2) and 2.5000% (C1) toxicant concentrations.

TABLE III
VIABILITY OF SPERM CELLS INDUCED BY DIFFERENT CONCENTRATIONS OF CAC₂ IN ALBINO MICE (*M. MUSCULUS*) AFTER 5 WEEKS OF EXPOSURE

% toxicant concentration /20 g of feed	Replicates	Total number of sperms scored	Number of viable sperms	% Viability
0.0000%	CCA	800	728	91
	CCB	800	760	95
	CCC	800	720	90
				(mean=92.0)
0.3125%	C4A	800	544	68
	C4B	800	640	80
	C4C	800	688	86
				(mean=7.8)
0.6250%	C3A	800	576	72
	C3B	800	560	70
	C3C	800	584	73
				(mean=71.7)
1.2500%	C2A	800	480	60
	C2B	800	496	62
	C2C	800	512	64
				(mean=62.0)
2.5000%	C1A	800	480	60
	C1B	800	440	55
	C1C	800	520	65
				(mean=60.0)

IV. DISCUSSION

The toxicant appeared to induce slight weight gains, especially in the organisms exposed to higher concentrations of the chemical after five week exposure period. Weight is often used as a vital sign of health status of organisms. According to [10], while there appear to be a consensus among scientists and clinicians that body weight loss reduces the risk of several chronic diseases, this apparently favourable effect should be balanced against any potentially harmful side effects of weight loss. They further observed that weight loss produced an increase in blood concentration of potentially toxic organochlorine pollutants in animals that can cause elevated plasma and subcutaneous adipose tissue concentrations of the pollutants in obese subjects.

The morphological abnormalities induced in sperm cells of the model organism included Double Head, Pin Head, Knobbed Head, No Tail, and With Hook malformations. These were mostly associated with the higher concentrations of the toxicant, and the With Hook abnormality was proportionally higher and consistent over all other varying types of sperm head abnormalities in the mice. Several authors have also recorded abnormalities in sperm cells of mice exposed to environmental toxicants, including [11] on induction of abnormal sperm morphology in mice by some anthelmintic drugs, [12] on the evaluation of induction of abnormal sperm morphology induced by landfill leachates on mice, [8] on the mutagenic effects of Praziquantel on sperm heads of the mice, and [9] on morphological inductions of

sperm cells near a radio frequency radiation source proximal to residential quarters.

The sperm cells with no tails could have been the result of tails that cut off from the head during experimental preparation and/or storage. The other abnormalities could be due to the induction of point mutations in the early spermatocytes and spermatogonia at the pre-meiotic stages of spermatogenesis [8]. This thus confirms inductions from toxicant exposure, since spermatogenesis in mice normally lasts a period of 35 days (5 weeks) [13]. Further, [14] reported that exposure to chemicals could produce pituitary-hypothalamic or sex hormonal effects which in turn could affect spermatogenesis or cause abnormalities in seminal fluid resulting in functional or structural impairment of the sperm cells of test organisms. In his work on biology of fertilization, [15] states that one of the most enduring needs of reproductive biology is to understand the fundamental cellular mechanisms that control the fertilizing potential of human spermatozoa. Earlier, [16] asserted that it is known that defective sperm function remains the single largest defined cause of male infertility. Moreover, it is also clear that male infertility has a multi-factorial origin including genetics, environment, diseases and iatrogenic.

The morphological abnormalities observed among the control mice used in the current work could be as a result of natural mistakes in spermatozoon differentiating process and abnormal chromosome complement, an observation also made by [12].

The different toxicant concentrations also caused inductions in sperm cells viability. Obviously, the sperm cells from the control mice were most viable than those exposed to the toxicant concentrations. This compliments observations earlier made with more sperm cell abnormalities associated mainly with the higher toxicant concentrations. Consequently, higher toxicant dosing made sperm cells from the model organisms less viable than those from control group. The implication of this on the reproductive potential of the organisms could range from low birth to outright infertility in the larger mammalian population. Adami et al. [17] asserted that the importance of possible decline in semen quality lies partly in its possible link with other problems of male reproductive organs, such as a widespread rise in the incidence of testicular cancer, and a suggested increase in hypospadias and cryptorchidism in some geographical areas.

V. CONCLUSION

This research revealed slight increase in weights of mice exposure to CaC_2 for 5 weeks. The common abnormalities induced in the sperm cells of the mammal included Double Head, Pin Head, Knobbed Head, No Tail, and With Hook. Sperm cells in the control experiment were more viable than those in the treatment setup.

ACKNOWLEDGMENT

The authors are grateful to Dr. Ujowundu of the Department of Biochemistry, Federal University of Technology Owerri for

providing some materials used in this study. We are also indebted to Ejimedicals Diagnostic Laboratories in Owerri, for their assistance in semen analysis.

REFERENCES

- [1] M. Asif, "Physico-chemical Properties and Toxic Effect of Fruit Ripening against Calcium Carbide," *Annals of Tropical Medicine and Public Health*, 2012, vol. 5, pp. 150-160.
- [2] Consumers Association of Penang (CAP) (2014), "Ban the Use of Carbide to Ripen Fruits", Retrieved July 20, 2014 from <http://www.consumers.org.my/index.php/food/safety/502>
- [3] S.A. Fattah, and M.Y. Ali, "Carbide Ripened Fruits -A Recent Health Hazard", *Faridpur Medical College Journal*, 2010, vol. 5. No. 2, p. 37.
- [4] USA Department of Health, "Description of a Hazardous Substance", *Fact Sheet*, USA: Trenton, NJ. 2000, pp. 1-4.
- [5] H. Kjuus, A. Andersen, and S. Langard, "Incidence of Cancer among Workers Producing Calcium Carbide", *Porsgrunn and the Cancer Registry of Norway*, Norway, 2007.
- [6] W.S. Rahman, and M.B. Chowdhury, "Artificial Ripening: What we are Eating", *Journal of Medicine*, 2008, vol. 9, pp. 42-44.
- [7] A.J. Dhembare, A.B. Gholap, and K. Vandana, "Bitter Truth About Artificial Ripener" *Journal of Experimental Zoology India*, 2011, vol. 14, no. 1, pp. 187-189.
- [8] R.K. Aduloju, O.A. Otubanjo, and P.G.C. Odeigah, (2008) "An *in vivo* assay of the mutagenic potential of praziquantel (PZQ) using sperm head abnormality test", *Journal of Human Ecology*, 2008, vol. 23, no. 1, pp. 59-63.
- [9] A.A. Otitolaju, I.A. Obe, O.A. Adewale, O.A. Otubanjo, and V.O. Osunkalu, "Preliminary study on the induction of sperm head abnormalities in mice, *Mus musculus*, exposed to radiofrequency radiations from Global System for Mobile Communication base stations", *Bull. Environ. Contam. Toxicol.*, 2010, vol. 84, pp. 51-54.
- [10] J. Chevrier, E. Dewailly, P. Ayotte, P. Mauriege, J.P. Despres, and A. Tremblay, "Body Weigh Los Increases Plasma and Adipose Tissue Concentrations of Potentially Toxic Pollutants in Obese Individuals" *International Journal of Obesity*, 2000, vol. 24, no. 10, pp. 1272 – 1278.
- [11] O.A. Otubanjo, and A.A. Mosuro, "An *in vivo* evaluation of induction of abnormal sperm morphology by some anthelmintic drugs in mice." *Mutat. Res.*, 2001, vol. 497, pp. 131-138.
- [12] A.A. Bakare, A.A., Mosuro, and O. Osibanjo, "An *in vivo* evaluation of induction of abnormal sperm morphology in mice by landfill leachates. *Mutat Res*, 2005, vol. 582, pp. 28-34.
- [13] W.R. Bruce, and J.A. Heddle, "The mutagenic activity of 61 agents as determined by the micronucleus, *Salmonella* and sperm abnormality assays", *Canadian Journal of Genetics Cytology*, 1979, vol. 21, pp. 319-334.
- [14] P.G.C. Odeigah, "Sperm head abnormalities and dominant lethal effects of formaldehyde in albino rats" *Mutation Research*, 1997, vol. 389, pp. 141-148.
- [15] R.J. Aitken, "The Biology of Fertilization", *Advance in Experimental Medicine and Biology*, 1997, vol. 424, pp. 29-291.
- [16] M.G. Hull, "Infertility", *Practitioner*, 1985, vol. 229, pp. 5-943.
- [17] H.O. Adami, R. Bergstrom, and M. Mohner, "Testicular Cancer in Nine Northern European Countries. *International Journal of Cancer*, 1994, vol. 59, pp. 33-38.