EFFECT OF EXPOSURE TO TYRE SMOKE ON HACMOGLOBIN SERUM CALCIUM AND ALBUMIN OF SELECTED ABATTOIR WORKERS IN TRANS – AMADI SLAUGHTER OF PORT HARCOURT.

ABSTRACT
This investigation was conducted to study the effect of exposure to tyre smoke on serum calcium and albumin of selected abattoir workers in Oginigba town of Obio area of Port Harcourt, Rivers State. A total number of 50 participants were selected for the study (35 experimental group and 15 controls). Blood samples were collected by venipuncture and analyzed in the laboratory for serum calcium and albumin using the colorimetric and bromo cresol-green method respectively. Compared to 3% of the control population, 49% of the experimental population had a serum calcium level below the standard reference of 2.20-2.55mmol/l. Results from the test on serum albumin showed that 23% of the experimental population had level above the standard references of 3.5-50g/dl whereas the control population had values within the reference range. The experimental population was grouped into five study groups: 1-3, 4-6, 7-9, 10-15, and above 15 years of service (exposure years) at the abattoir. Serum calcium levels in mmol/l were 2.31, 2.15, 2.20, 2.13, 2.06, 2.16, and serum albumin level in g/dl were 4.63, 4.62, 4.70, 4.79, 4.83, 4.99 for control, groups 1-3, 4-6, 7-9, 10-15 and above 15years respectively compared with the control, all showed significant increase in the serum albumin levels. The level of significance in agreement with the different standard reference ranges, serum calcium was observed to significantly vary from the control from the last level of exposure (1-3years) while serum albumin differed significantly from the control, from 7years of exposure for non-smokers and 4years of exposure amongst smokers. The data was group into two study based on genotype as AA and AS. It was observed that participants with genotype AS were most susceptible to the effect of smoke inhalation on lowering serum calcium levels. The result of this research showed that the abattoir workers (roasters) are prone to developing hypocalcaemia and hyperalbuminaemia, and therefore require periodic medical attention.

INTRODUCTION
From several studies, it has been established that exposure to smoke poses a challenge to human health at environmental relevant concentrations. Some effect associated with wood smoke are not unlike those of mixed urban ambient particulate matter(PM) for both cancer and non-cancer end-points(Le Van *et al.,*2006). Smoke from wood burning has some unique component but also shares many physical and chemical characteristics with emissions from other combustion source (Bergauffet *et al.,* 2008). The effect, exposure concentrations and chemical profiles are expected to vary significantly depending on specific scenarios and human receptors (Larson *et al.,* 2007).

At emission, wood smoke consists of solids, liquid and gaseous constituents that change and sometimes rapidly with time, temperature, sunlight, and interaction with other pollutants and water surfaces. Many of these constituents are known to be hazardous to human health but are not specifically regulated or even fully evaluated (Nacher *et al.,* 2007).

Organic extracts of ambient particulate containing substantial quantities of wood smoke are 30_ fold more potent than extract of cigarette smoke condensate in a mouse skin tumor indiction assay (Nacher *et al.,* 2007). Thirty seven percent (37%) of lung cancer patients had an association with wood smoke exposure(Delgado *et al.,* (2005). Among non-smokers, out of all the cooking fuels, the risk of development of lung cancer was highest for biomass fuel exposure (Behera & Balamugesh, 2005). Burning wood in the kitchen increases the risk of cervical neoplasia in HPV – infected women in Honduras (Velema *et al.,* 2002). The use of biomarkers to assess wood smoke exposure is promising, yet there are limitations for its use as quantitative indicators of exposure (clark *et al.,* 2007).

Tyre combustion produces particulate matters which are aggregates of small particulates, liquid droplets and vapours. The particulates are of aerodynamic diameter PM2.5 and PM10. Tyre combustion results into volatiles which undergo degradation to produce free radicals considered biologically hazardous (Bosire etal., 2016). These can represent both significant short term and long term hazards to individuals and the environment (Tsai, 2016). Emissions from burning of tyres are associated with serious public health ailments including whizzing of the lungs and chronic coughs. Emissions from burning tyres are highly mutagenic and carcinogenic (Downward *et al.,* 2015).

Open tyre fire emissions are estimated to be 16 time more mutagenic than residential wood combustion in a fire place and 13000 time more mutagenic than coal fired utility emission with good combustion efficiency and add-on controls (Reisman, 1997).

The objectives of the study were to: evaluate the effect of inhalation of tyre emission on serum calcium and albumin levels of the 'roasters', determine a safe exposure limit to the fire points (in service as a roaster) in years, evaluate the added effect of cigarette smoking on human serum calcium and albumin level of the 'roasters and evaluate the influence of the different hemoglobin variants to the effect of the smoke inhalation.

MATERIALS AND METHODS
Sample population and size
A total number of 50 individuals participated in this study. Thirty – five (35) participants form the experimental population while the remaining 15 participants serve as the control population. Both the experiment and control populations are inhabitants of Port Harcourt, Rivers State. The experimental populations were selected from the carcass handlers (roasters) at the abattoir while the control populations were selected outside the abattoir (none abattoir workers).

Sample Collection and Preparation

Each participant's name, age, sex, length of service at the abattoir, health history and smoking habit were noted down and each participant's sample bottle appropriately labeled. The tourniquet was placed on the arm about four (4) inches above the vein of interest. The area was thoroughly cleaned and disinfected with 70% alcohol by at least 2cm by 2cm for 1 minute and allowed to dry. The blood sample was collected and transferred immediately into vacationer bottles for laboratory examination. The blood was allowed to clot a room temperature. Clot was removed by centrifuging and the supernatant (serum) was transferred immediately into a clean polypropylene tube using a pipette and maintained at 2-8^0c while handling.

Comment [SZ3]: gender

Biochemical Assay

Calcium test was conducted using the colorimetric method while bromocresol – green method was used to conduct albumin test. Curettes (1cm light path), spectrophotometer, and test tubes were used for both tests while water bath was used for albumin test only. Hemoglobin electrophoresis was carried and cellulose acetate paper, whatman's filter paper, and electrophoresis machine were the materials used.

RESULTS

The results obtained for the serum calcium and albumin of 35 healthy participants selected from the abattoir of the 'roasters' community with the code number, age, level of exposure and smoking of the Texperimental population, and also of 15 male healthy participants who do not smoke selected outside the abattoir are shown in Tables 1and 2 respectively.

Comment [SZ4]: The results should be interpreted properly. In a descriptive format

Table1: Serum albumin and calcium concentration of male experimental population

Serial no	Calcium (mmol/l)	Albumin (g/dl)	Genotype	Age (years)	Exposure in years	Smoke
1	2.10±0.00	4.58±0.01	AS	38	3	NO
2	2.18±0.00	4.85±0.00	AA	37	3	NO
3	2.21±0.01	4.76±0.00	AA	25	8	YES
4	1.92±0.00	5.03±0.01	AA	26	6	YES
5	2.22±0.00	4.60±0.01	AA	20	3	NO
6	2.15±0.01	4.93±0.00	AA	29	15	NO
7	2.28±0.02	4.68±0.00	AA	23	10	NO
8	2.26±0.00	4.06±0.00	AS	22	4	NO
9	2.27±0.00	5.28±0.02	AA	21	5	NO
10	2.29±0.00	4.56±0.00	AS	20	2	NO
11	2.31±0.01	4.90±0.00	AA	21	4	NO
12	2.37±0.00	5.13±0.00	AA	37	19	NO
13	2.30±0.00	5.02±0.00	AA	40	17	NO
14	2.16±0.03	4.47±0.01	AS	24	4	NO
15	2.04±0.01	4.79±0.01	AS	30	2	NO
16	2.32±0.00	5.15±0.01	AA	40	5	YES
17	1.74±0.03	3.65±0.00	AA	37	5	NO
18	1.81±0.00	4.83±0.03	AA	42	27	NO
19	2.21±0.02	5.35±0.00	AA	40	5	NO
20	2.14±0.01	4.61±0.00	AA	45	7	YES
21	2.42±0.01	4.65±0.01	AS	46	10	NO
22	2.47±0.00	4.57±0.00	AA	27	4	NO
23	1.99±0.03	4.29±0.00	AA	25	2	NO
24	2.11±0.01	4.85±0.00	AS	34	7	NO
25	2.21±0.00	4.90±0.02	AA	45	11	NO
26	2.24±0.02	4.45±0.01	AA	28	4	NO
27	2.02±0.00	4.88±0.00	AS	35	12	NO
28	2.27±0.00	4.47±0.02	AA	29	4	NO
29	1.98±0.01	4.76±0.00	AS	42	11	NO
30	1.79±0.01	4.68±0.00	AS	42	10	NO
31	2.22±0.00	5.12±0.00	AA	39	5	YES
32	2.08±0.00	4.90±0.04	AA	32	9	NO
33	2.12±0.00	4.83±0.01	AA	36	7	NO
34	2.22±0.01	4.68±0.01	AA	39	3	NO
35	1.87±0.00	5.14±0.00	AS	41	10	NO

Calcium and albumin values are means of 3 determinations ± standard deviation.

Table 2: Serum albumin and calcium concentration of the control population.

Serial no	Calcium (mmol/l)	Albumin (g/dl)	Age (years)
1	2.29±0.01	4.71±0.00	30
2	2.29±0.00	4.72±0.01	24
3	2.19±0.00	4.66±0.01	38
4	2.23±0.02	4.55±0.00	20
5	2.45±0.01	4.75±0.01	23
6	2.37±0.00	4.69±0.00	30
7	2.40±0.00	4.71±0.00	37
8	2.35±0.01	4.40±0.01	29
9	2.50±0.00	4.59±0.00	24
10	2.32±0.03	4.57±0.00	34
11	2.09±0.01	4.55±0.00	20
12	2.16±0.00	4.58±0.01	22
13	2.37±0.00	4.77±0.00	31
14	2.28±0.00	4.68±0.00	26
15	2.34±0.00	4.56±0.00	33

Calcium and albumin values are means of 3 determinations ± standard deviation.

The experimental population was grouped into five classes based on the years of service at the abattoir namely 1-3,4-6,7-9-15 and 16 years upwards, descriptive statistic and one sample T-test were employed to compare the means against the control and to test for significance at 95% confidence level; and the experimental population of 35 participants grouped into genotype classes AA and AS, 11 of which are of genotype AS which are of genotype AA are seen in Tables 3and respectively

Table 3:   Result of mean ± standard deviation of serum calcium and albumin with respect to exposure levels

Exposure in years	Calcium (mmol/l)	Albumin (g/dl)
Control	$2.31^a \pm 0.11$	$4.63^a \pm 0.10$
1-3	$2.15^b \pm 0.11$	$4.62^a \pm 0.18$
4-6	$2.20^{ab} \pm 0.19$	$4.70^{ab} \pm 0.51$
7-9	$2.13^b \pm 0.05$	$4.79^b \pm 0.11$
10-15	$2.06^b \pm 0.17$	$4.83^b \pm 0.17$
16 & above	$2.16^{ab} \pm 0.31$	$4.99^a \pm 0.15$

Values are means ± standard deviation. Means bearing different superscript on one column are significantly different ($P<0.05$).

Table 4:   Serum calcium and albumin concentration with respect to genotype

Table 4.7.1: Serum calcium and albumin concentration with respect to genotype

Serial no	GENOTYPE AS		AA	
	Calcium (mmol/l)	Albumin (g/dl)	Calcium (mmol/l)	Albumin (g/dl)
1.	2.10±0.00	4.58±0.01	2.18±0.00	4.85±0.00
2.	2.26±0.00	4.06±0.00	2.21±0.01	4.76±0.00
3.	2.29±0.00	4.56±0.00	1.92±0.00	5.03±0.01
4.	2.16±0.03	4.47±0.01	2.22±0.00	4.60±0.01
5.	2.04±0.01	4.79±0.01	2.15±0.01	4.93±0.00
6.	2.42±0.01	4.65±0.01	2.28±0.02	4.68±0.00
7.	2.11±0.01	4.85±0.00	2.27±0.00	5.28±0.02
8.	2.02±0.00	4.88±0.00	2.31±0.01	4.90±0.00
9.	1.98±0.01	4.76±0.00	2.37±0.00	5.13±0.00
10.	1.79±0.01	4.68±0.00	2.30±0.00	5.02±0.00
11.	1.87±0.00	5.14±0.00	2.32±0.00	5.15±0.01
12.			1.74±0.03	3.65±0.00
13.			1.81±0.00	4.83±0.03
14.			2.21±0.02	5.35±0.00
15.			2.14±0.01	4.61±0.00
16.			2.47±0.00	4.57±0.00
17.			1.99±0.03	4.29±0.00
18.			2.21±0.00	4.90±0.02
19.			2.24±0.02	4.45±0.01
20.			2.27±0.00	4.47±0.02
21.			2.22±0.00	5.12±0.00
22.			2.08±0.00	4.90±0.04
23.			2.12±0.00	4.83±0.01
24.			2.22±0.01	4.68±0.01

Calcium and albumin values are means of 3 determinations ± standard deviation

Correlation between the experimental, control and reference ranges of serum calcium and albumin are seen in figures 1 and 2 respectively.
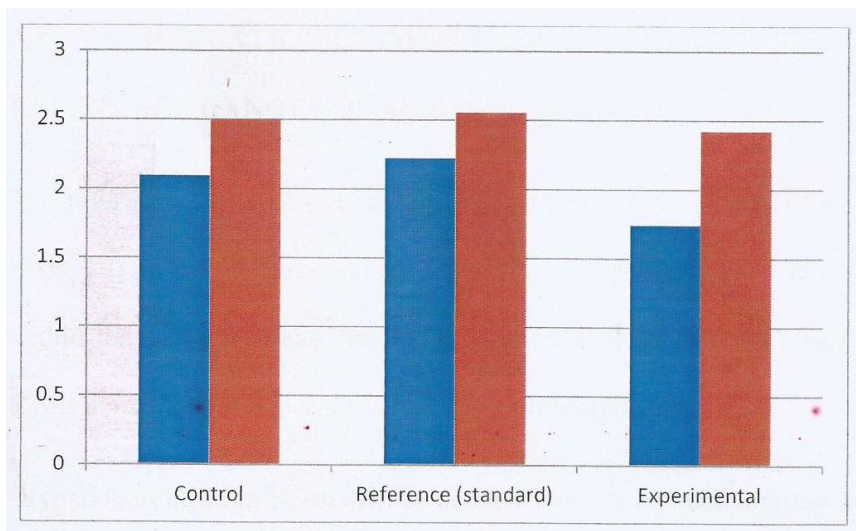

Figure 1: Serum calcium representation of both experimental, control and reference (standard) showing the minimum and maximum points for each.
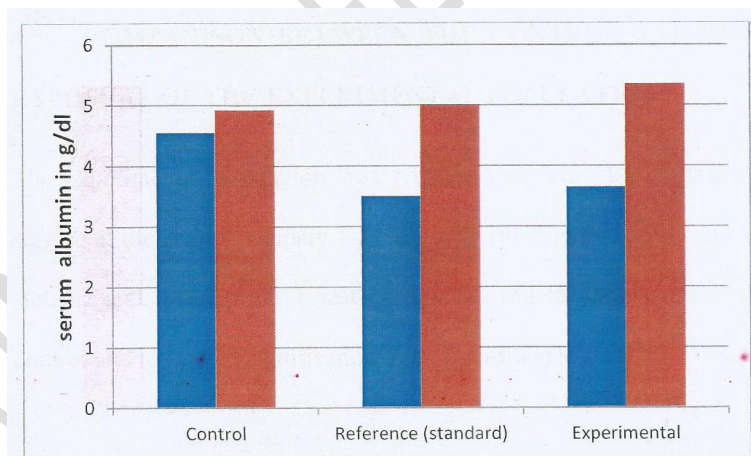

Figure 2:   Serum albumin representation of both experimental, control and reference (standard) showing the minimum and maximum points for each.

Comparison between the control and the levels of exposure for serum calcium and albumin are shown in figures 3 and 4 respectively.
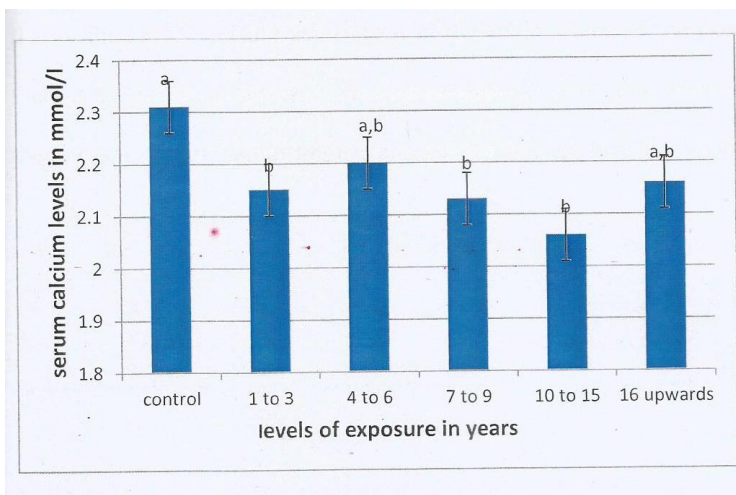
Figure 3:   Serum calcium mean values of the exposure levels. Bars bearing different letters are significantly different at p≤ 0.05
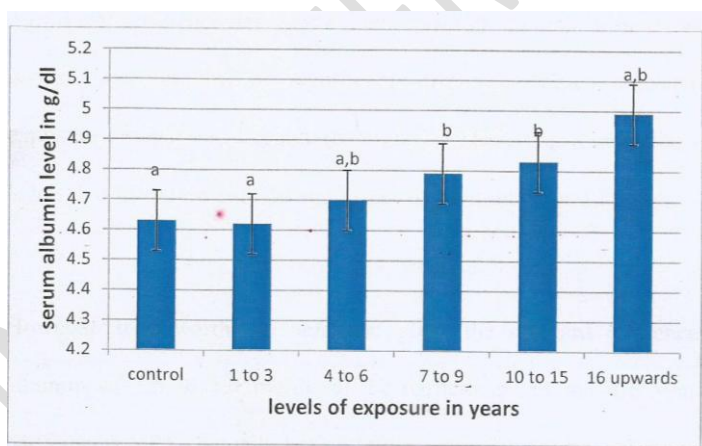

Figure 4:   Serum albumin mean values of the exposure levels. Bars bearing different letter are significantly different at p≤ 0.05

DISCUSSION

The reference range for normal serum calcium is 8.8-10.2mg/dl equivalent to 2.20-2.55mmol/l (Stephen & Karen, 2006). Of the experimental population of 35 participants, 18(51%) are within the reference range and 17(49%) are below the reference range. The reference range for normal serum albumin is 3.5-5.0g/dl (Burtis *et al.,* 2012). Of the experimental population of 35 participants 8(23%) are above the reference range (of these, 9% are cigarette smokers) and 27 (77%) are within the reference range.

The lower calcium levels could be as a consequence of lower absorption of calcium and vitamin D reported among smokers (Need *etal.,* 2002). Hypocalcaemia could also arise as a result of deficiency in parathyroid hormone (PTH) which can be a result a mutation in the calcium-sensing receptor caused by the inhalation of smoke constituents (Skugor, 2014). Amidst the consequences of hypocalcaemia which include seizures, impaired intellectual capacity, cardiac arrest, numbness, muscle spasms etc., it is also possible to be asymptomatic especially when it develops slowly leading to a fatal stage (Cooper & Gittoes, 2008) hyper albuminaemia is usually associated with abrupt deghydration and high protein diets (Busher, 1990) Mutlu *etal.,* 2006). Even though serum albumin is a protein with antioxidant activity, it is also implicated with an increased risk of cancer of the distal colon (Knekt *et al*. 2000).

Approximately, 40% of total plasma calcium circulates bound to albumin. When circulating albumin is reduced, total serum calcium must be adjusted to reflect lower amounts of bound calcium. More specifically each 1g/dl decrease of album will decrease 0.8mg/dl in measured serum calcium. Thus, 0.8 must be added to the measured calcium to get a corrected calcium value (Fox, 2017). Several disorders of calcium sensing arise from inherited or acquired abnormalities that 'reset' the serum calcium concentration upwarders or downwards (Egbuna & Brown, 2008)

Earlier research involving sickle cell trait (hemoglobin genotype AS) revealed that sickle cell trait has a protective advantage against *Plasmodium falciparum* (Williams *et al.,* 2005). However, there are rare complications associated with it. Exercise induced dehydration exhaustion may cause healthy red blood cells to turns sickle-shaped which can cause death during sporting activities (Eichner, 2007). Sickle cell disease and trait erythrocytes have been reported to show alterations in sensitivity to elevated intracellular calcium levels (Judd et al., 2003).

Conclusion

In conclusion, exposure to smoke either from tyre or wood origin has been shown to affect health in varied ways (Larson *et al.,* 2007, Le-Van *et al.,* 2006). It is evident from the research conducted that some of the carcass handlers (roasters) should be recommend for periodical medical checks.

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REFERENCES

Behera, D.& Balamugesh, T. (2005). Indoor air pollution as a rish for lung cancer in women. Journal of the Association of Physicians in India, 53,190-192.

Bergauff, M., Ward, T., Noonan, C.(2008). Determination and evaluationof selected organic chemical tracers for wood smoke in airborne particulate matter. International Journal of Environmental Analytical Chemistry,88(7),473 -486.

Bosire, J., Kibet J., Kinyanyui, T.,& Githaiga, B. (2016). Tire combustion emissions and their histochemical implication ofn the lung tissues of albino mice. Open Access Library Journal, 3(10),1-14.

Burtis, C. A., Ashwood, E.R., & Brun, D.E. (2012). Tietz textbook of chemical chemistry and molecular diagnosis (5th edition). St. Louis, USA: Elsevier.

Busher, J.T. (1990). Serum albumin and globulin. In Walker, H.K., Hall W.D.,& Hurst, J.W.(Editors), clinical methods: the history physical and laboratory examinations (3rd edition). Emory University School of Medicine, Atlanta, Georgia. Boston: Butter worth's.

Clark,M.,Paulsen, M., Smith, K.R., Canuz, E., & Simpson, C.D. (2007). Urinary methoxyphenol biomarkers and wood smoke exposure: comparisons in rural Guatemala with personal CO and Kitchen CO, levoglucosan and $PM_{2.5}$ Environmental Science & Technology,41(10),3481-3487.

Delgado,J., Martinez, L., Ramirez, A., Lturrai, C., & Gonzalez-Avila,G.(2005). Lung cancer pathogenesis associated with wood smoke exposure. American college of chest physicians, 128,124-131.

Downward, J., Singh, A., Bullard, R., Jayarathne, T., Rathnayake, C.M., Simmons, D.L. Wels, B.R., Spak, S.N.,T.,& Beardsley D.(2015).Uncontrolled combustion of shredded tyres in a landfill. Atmospheric Environment, 014, 195-204.

Egbuna,O.I. & Brown, E.M. (2008) Hypocalcaemia and hypocalcaemia conditions due to calcium sensing receptor mutations. Best practice and research clinical rheumatology, 22(1), 129-148.

Eichner, E.R.(2007). Sickle cell trait. Journal of sports rehabilitation, 16(3), 197- 203.

Fox, M.(2017). High calcium and albumin. Livestrong. Retrieved from http://www. Livestrong.com

Judd, A.M., Best, K.B., Christensen K., Rodger, G.M., & Bell, J.D.(2003). Alterations in sensitivity to calcium and enzymatic hydrolysis of membranes from sickle cell disease and trait erythrocytes, American Journal of Hematology, 72(3), 162-169.

Knekt. P., Hakulinen, T., Leino, A, Heliovaara, M., Reunanen, A.,& Stevens, R. (2000). Serum albumin and colorectal cancer risk. European Journal of Clinical Nutrition, 54(1), 460-462

Larson, T.,Sun J., Baribeau, A.M., Buzzelli, M., Setton, E.,& Braver, M.(2007). A spatial model of urban winter wood smoke concentrations. Environmental Science & Technology, 41(7), 2429-2436.

Le Van, T.D., Koh, W. P., Lee, H.P., Koh, D., Yu,M.C.,& London, S.J.(2006). Vapour, dust and smoke exposure in relation to adult onset asthma and chronic respiratory symptoms – The Singapore Chinese health study. American Journal of Epideminology, 163(12), 1118-1128.

Mutlu, E.A., Keshavarzian, A.,& Mutlu, G.M.(2006). Hyperalbuminaemia and elevated transaminases associated with high – protein diet. Scandinavian Journal of Gastroenterology, 41(6), 759-760.

Nacher, L.P., & Braver, M., Lipsett, M., Zelikoff, J.T., Simpson C.D., Koenig, J.Q., & Smith, K.R. (2007). Wood smoke health effects: A review .Inhalation Toxicology, 19,67-106.

Need, A.G., Kemp, A., Giles, N., Morris, H.A., Horowitz, M., & Nordin, B.E.(2002). Relationships between intestinal calcium absorption, serum vitamin D metabolites and smoking in post menopausal women. Osteoporosis international, 13(1),83-88.

Reisman, J.J. (1997). Air emissions from scrap tire combustion. Environment protection Agency, Boo(97), 115-119.

Skugor, M. (2014).Hypocalcemia. Cleveland clinic. Retrieved from http://www.clevelandclinicmeded.com/medicalpubs/diseasemanagement/endocrinology/hypocalcemia

Stephen, E.L. & Karen, E.M. (2006). Compilation of NIST Higher Order Methods for the determination of electrolytes in clinical materials. National Institute of Standards & Technology, USA special publication, 260 (162), 1-102.

Tsai, W.T. (2016). Toxic volatile organic compounds (VOC$_S$) in the atmospheric environment. Regulatory Aspects and Monitoring in Japan and Korea, Environments, 3(3), 1-23.

Velema, J.P., Ferrara, A., Fiqueroa, M., Bulnes, R., Toro, L.A., De Barahona, O., Claros, J.M., & Melchers, W.J (2002). Burning wood in the kitchen increases the risk of cervical neoplasia in HPV-infected women in Honduras. International Journal of Cancer, 97(4), 536-541.

Williams, T.N., Mwangi, T.W., Wambua, S., Alexander, N.D. Kortok, M., Snow, R.W., & reMarsh, K. (2005). Sickle cell Trait and the Risky of plasmodium falciparum Malaria and other childhood diseases. The journal of infectious Diseases, 192 (1), 178-186.

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