PSYCHOPHARMACOLOGICAL EFFECT OF CYANIDE INGESTION ON PREGNANT RATS AND THEIR LITTERS

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ABSTRACT

Cyanide is responsible for the degenerative effect of certain brain areas responsible for some behavioural outcomes in animals and humans alike. Though cyanide exists generally in the environment, it is also commonly contained in some natural food sources notably, vegetables, fruits, and root crops, like cassava, a staple food in Nigeria and other tropical countries. This study, therefore, investigated the effects of chronic ingestion of cyanide on pregnant rats and their offspring. A total of 60 Wister Albino rats were used in the study. They comprised of 20 female pregnant rats that ingested cyanide and 40 of their offspring that were prenatally exposed to cyanide. The 20 pregnant female rats were randomly assigned into four treatment groups of five rats each. Group 1 was given low dosage cyanide (0.1mg/kg body weight), group 2 was given high dosage cyanide (0.4mg/kg body weight), group 3 received saline, and group 4 received no treatment, while 10 of their offspring were selected from each treatment group from a total population of 103 offspring littered by the pregnant rats. Treatment covered the first 16 days of the gestation period. Dependent variables observed were the psychopharmacological outcomes of cyanide ingestion such as body weight maintenance of the mother rats, infant mortality, Litter size, average birth weight, still birth, malformation, and exploratory learning behaviour of the offspring. The analysis was done using independent group randomized design and tested four hypotheses using one-way analysis of variance and the independent t-test. Results showed that Mother rats that received a high dosage of cyanide gained less weight when compared with Mother rats in other treatment groups ($F_{(316)} = 21.18$, $P<.01$). Infant mortality was found to be higher among the offspring of rats that received a high dose of cyanide ($F_{(2.4)} = 26.88$, $P<.05$), when compared to offspring of rats that were treated with a low dosage of cyanide. Infant mortality was not observed in either the saline or the "no treatment" groups. Exploratory learning behaviour of offspring of rats prenatally exposed to cyanide was significantly affected ($F_{(3.36)} = 14.35$, P.001). Offspring of rats prenatally exposed to the high dose of cyanide were slower in learning tasks, ($\bar{x} = 212.43$) than those given low dose of cyanide ($\bar{x} = 147.54$). However, there was no significant effect on the growth of offspring of rats prenatally given cyanide ($t = 0.58$, $df=15$, $P>.05$). It is therefore concluded that chronic ingestion of cyanide by pregnant mother rats has a negative effect on both the mother rats and its Litters, more so during the neonatal period. A recommendation is hereby made to the government for enactment and enforcement of enabling laws and policies aimed at eliminating or reducing the concentration of cyanide in foods as beneficial effects may be observable in human populations.

***Keywords**: Cyanide, Litters, Psychopharmacological, Pregnant Rats*

INTRODUCTION

Rapid development and industrialization by man have contributed negatively to the disturbance of the delicate ecological balance in the environment. Efforts are deliberately made in developed countries to prevent deterioration or transformation of the natural environment, but this is not so in developing countries. Cyanide used for different purposes by man is one of the major causes of environmental deterioration.

Cyanide has created complex problems for modern society, and these problems have evolved mainly from industrial pollution. Its use as suicidal, chemical warfare and genocidal agent are well known (Sykes, 1981). Toxic problems have been associated with ingesting cyanide-containing foods and occupational hazards have arisen as the industrial use of cyanide has increased. The major target site for cyanide is the brain, where potentially lethal cytotoxic hypoxia is induced (Ballantyne, 1975). Hypoxia, however it is caused, results in a decreased supply of oxygen at the cellular level and alteration in behavioural profiles, most importantly in relation to memory consolidation. As the intensity and duration of cytotoxic hypoxia increases in the brain, the probability of secondary insult via vascular and respiratory complications also increases (Brierley et al., 1977).

Cyanides comprise a wide range of compound of varying degree of chemical complexity, all of which contain a CN moiety, to which human are exposed in gas, liquid and solid form from a

broad range of natural and anthropogenic sources. While many chemical forms of cyanide are used in industrial application or are present in the environment, the cyanide anion (CN-) is the primary toxic agent, regardless of origin.

Principal natural sources of cyanide are over 2000 plant species including fruits and vegetables that contain cyanogenic glycoside which can release cyanide on hydrolysis when ingested. Among them, cassava (tapioca, manioc) is a staple food for hundred millions of people in many tropical countries including Nigeria. Known cyanogenic glycosides in the plant include amygdaline, linamarin, prunasin, etc. Hydrogen cyanide is also released into the atmosphere from natural biogenic processes from higher plants, bacteria, and fungi. In the air, cyanide is present as gaseous hydrogen cyanide with a small amount present in fine dust particles. Cyanides have the potential to be transported over long distances from their respective emission sources.

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Many edible plants contain cyanogenic glycosides whose concentrations can vary widely as a result of genetic and environmental factors, location, season and soil types (JECFA, 1993). Cassava, a tropical foodstuff contains a very high amount of cyanogenic glycoside compared to other edible plants. Bitter cassava dried root contains 2360 mg/kg cyanogenic glycoside, cassava bitter leaves contain 300mg/kg cyanogenic glycoside, cassava bitter whole tubers contain 380mg/kg cyanogenic glycoside and garri flour (Nigeria) contain 10.6-22.1 mg/kg cyanogenic glycoside compared to other edible plants such as cereal grains containing 0.001 - 0.45 cyanogenic glycosides, soy protein products containing 0.07-0.3 mg/kg cyanogenic glycoside, etc. (Narty 1980, Honig et al 1983; JECFA, 1993, ATSDR 1997).

The effect of dietary cyanide exposure from cassava is determined by the nutritional status of the individual (Delange, Ekpechi & Rosling 1994). The body needs about 1.2mg of daily supply of dietary sulphur to detoxify about 1.0mg of hydrocyanic acid (Padmaja 1996). If the demand of sulphur containing amino acids is prolonged as in the regular consumption of inadequately processed cassava and the diet is inadequate, the synthesis of many proteins vital for the body functions may be impaired leading to the development of protein deficiency disease (Padmaja 1996). In a situation where famine or extreme poverty may force a population to eat poorly processed cassava in a diet that is also deficient in nutrients such as protein, the plant cyanogenic glycoside can lead to poisoning. Examples are the infantile Kwashiorkor epidemic in the famine-stricken Biafra during the Nigeria civil war in 1968 and the reports of spastic paraparesis or Konzo in drought-stricken region of Mozambique and Tanzania (Padmaja, 1996; Howlett et al 1999; Milingi et al 1991; Akintonwa and Tunwase, 1992; Ernesto et al 2002; Sreeja, 2003).

Because of its effective detoxification mechanism, the cumulative effects of cyanide are rarely seen. For example, rats tolerated 25 daily doses of potassium cyanide, each of which was equivalent to the acute oral LD50 dose, when given in the diet(Hayes, 1967). Tewe and Maner (1982) studied pigs and rats to investigate the toxicological interrelationship of cyanide, dietary protein, and iodine and reported that there was no effect on body weight, food consumption and organ weights following cyanide administration.

Much of the toxicological interest in cyanide has been focused on its rapid lethal action; however, the most widespread problems arising from cyanide are from chronic dietary, industrial and environmental sources. As the major part of the exposed population is relatively poor, and are often also malnourished, especially in developing countries, adequate attention must be focused on the chronic effects of sublethal cyanide toxicity in malnourished individuals. But reports on the chronic toxicity of cyanide on brain neurotransmitters and behaviour are scanty.
Hence, this study aims to evaluate the chronic toxicity of cyanide at sub-lethal doses, in normal pregnant albino rats and their litters, on spatial memory and pharmacological effects to simulate a condition seen when people are ordinarily exposed to cyanide.

MATERIALS AND METHOD

DESIGN

Independent group randomized design was adopted for the study. The independent variables involved in this study were the cyanide treatment, saline treatment, and no treatment. The variables were manipulated at different treatment levels of low (0.1mg/kg body weight) and high (0.4mg/kg body weight) of cyanide, saline, and no treatment. The dependent variables are the psychopharmacological effects of cyanide on body weight of dams, the weight of pups, litter size, malformation of pups, and infant mortality and exploratory learning behaviour.

SUBJECTS

A total of 120 Young adult Sprague-Dawley white albino rats, 6-8 weeks old, weighing between 160-250g upon arrival were used. The rats were acquired from the Veterinary Animal House of the University of Ibadan, Nigeria, where animals for experimental studies are kept. These are the common breed and used a strain of rats in the laboratory and have also been extensively used in neurobiological research (Kuramoto *et al*. 2012). The animals were kept in rat cages in the experimental animal laboratory of the Department of Psychology, University of Ibadan, Nigeria. The rats were used according to institutional regulations on handling and efficient use of laboratory animals. They were made up as follows:
The females which were the mothers (dams), numbering 40 and weighing between 160 and 200 grams on the first day of the study, were randomly assigned into 4 equal treatment groups of 10 rats each to make up for the 4 separate independent groups. The treatment administered in the 4 separate groups is saline, no treatment, 0.1mg/kg cyanide and 0.4mg/kg cyanide. Out of the 40 female albino rats used for the study, 15 were discarded because they were found not to be pregnant and 5 died due to cyanide administration. Therefore, a total of 20 female albino rats, assigned to 5 female rats in each group was left for the study.
Male Wistar albino rats numbering 40 and weighing between 170 and 250 were used only as mates for the female rats. They were randomly assigned to one female rat each and housed in the same cage for three days during which conception was expected to take place. The mating was done at the ratio of 1:1 (one male rat to one female rat) to increase the chances that conception would take place.
The offspring of dams were randomly selected from each of the treatment groups for the purpose of learning behaviour of the offspring. For this category, 10 rats were randomly selected from each treatment group of saline, no treatment, 0.1mg/kg cyanide and 0.4mg/kg cyanide. The total number of offspring used was 40.

HOUSING OF SUBJECTS

The subjects were housed in the experimental animal laboratory of the Department of Psychology in a condition of the normal day-night cycle. The subjects were housed together in groups of 8 rats in the laboratory in RCI North Kent plastic breeding cages for a period of 14

days to quarantine them and to get them adjusted to the laboratory environment different from where they were brought from. The RCI cages have a dimension of 56cm x 40cm x 18cm.
After 14 days of quarantine the rats were housed in twos - one male and one female inside RI, North Kent Plastic Cages each with a dimension of 44cm x 28cm x 20cm. Male subjects were removed at the end of the third day while the female lived alone in their cages till the end of the gestation period. The female rats at parturition continued to live with their offspring in the RCI North Kent plastic cages in order to breast-feed them. After weaning their offspring, the dams were removed while the offspring lived together in the same cage until the learning behaviour experiment was conducted. The rats were fed with mouse cubes, a complete rationed diet prepared for rats. The RI North Kent plastic cages had a food compartment unit where the mouse cube was placed and water was given to the rats through the water bottle compartment of the cages. Food and water were made fully and freely available for the rats throughout the duration of the study.

EQUIPMENT/MATERIALS

The following instruments were used for the study.

1. Rat cages: These were RCI North Kent plastic cages measuring 56cm x 40cm x 18cm for breeding and general housing of subjects in groups and RI North Kent plastic cages measuring 44cm x 28cm x 20cm for data collection purposes.
2. Two weighing balances were used for the study. They were,
   (i) A Chatillon, New York. N.Y. 11415 weighing balance with the capacity of 500 X 2g. This was used for weighing rats, and
   (ii) A computerized Metler-Toledo weighing balance with a maximum capacity of 5kg and a minimum capacity of 0.01mg. This was used for weighing chemical substances that require very sensitive weighing balance because of tiny quality involved.
3. A 250ml graduated measuring cylinder was employed for measuring saline used to dilute cyanide.
4. Water bottles with stainless steel tunnels were used to provide drinking water the subjects.
5. An oral cannula was used for oral administration of cyanide and saline.
6. Disposable 5ml and 2ml syringes and needles were employed for the collection of cyanide and saline during the dilution process.
7. Electronic stop clocks were used for accurate time keeping during learning experiment.
8. Standard animal feed (mouse cubes) were used for feeding the subjects.
9. NaCN (CAS No. 143-33-9, 97.2% purity) were bought from Sigma-Aldrich (St. Louis, MO) and stored at room temperature, and normal saline.
10. A T-maze was employed for measurement of exploratory learning ability of the rats.

EXPERIMENTAL PROCEDURE

The study started with the mating of dams with males of the same strain for three days. On the first day of the experiment, the males were randomly assigned to 20 cages because their role in this study was for mating purposes only. The females, on the other hand, were weighed and randomly assigned to any of the following treatment groups;

(i) Control group with no treatment
(ii) Control group with saline treatment
(iii) 0.1mg/kg cyanide group
(iv) 0.4mg/kg cyanide group.

Each treatment group comprised of ten dams. After assigning dams into groups, each dam was dropped into a numbered cage already containing a male rat for purpose of mating.
The chemical substance, cyanide was then prepared into fresh solutions of 0.1mg/ml and 0.4mg/ml concentrations. The dams were then given oral administration of cyanide with the help of the oral cannula. They were allowed a period of 30 minutes before food and water were restored to them to ensure that the effect of the cyanide has begun. On the third day of the experiment, the male rats were removed, leaving only the females in their individual cages.
The procedure of treatment was repeated daily for 16 days of the gestation period, to ensure that:

(a) The administration covered the three trimesters of dams' gestational period which normally spans for a duration of 21, +1 days.
(b) Dams were not given any treatment on the days of parturition. On this basis, it could be claimed that treatment covered most days of gestation.

The dams were only weighed and observed from day 17 to parturition without any treatment. At parturition, litter size and mean birth weight was taken at the nearest experimental time. Dams that were observed not to be pregnant were removed from the experiment while data collection on body weight, infant mortality, a manifestation of deformity (malformation) continued on other dams and their offspring.
The offspring were allowed to breastfeed for 21 days. However, the offspring were weaned at the end of 28 days to ensure that the offspring had enough breast milk to safeguard them against infant mortality at that stage.
The next stage of the experiment continued with the offspring. The offspring from all the dams in each treatment group were gathered together into one cage. Ten pups, (5 males and 5 females were randomly selected from each treatment group and data collected on them for learning behaviour.
The study proceeded by housing each pup in individual cages properly labeled according to each treatment group. The pups in each treatment group numbered 1-10 were also given tail rings as representing 1-10 according to their serial arrangement as a form of identification for each pup to avoid mix-up during the training session for the learning experiment. On each experimental day, the pups which were deprived of food for 12 hours were given three pieces of training trials on the T-maze to discriminate between the goal box, the runway and the radial arm painted white or black as the case may be where the food is placed. The food was interchanged between the white and black arms to measure how well the pups had mastered the positioning of the food item. On each experimental session, the pups were timed with the stop clock to measure the time taken by the pups to move from the go box to the radial arm of the T-maze as a measure of their learning ability. The procedure was repeated every other day for 14 days. This was to ensure that the pups take enough food before the next experimental session when food would be taken away from them.

MEASUREMENT OF DEPENDENT VARIABLES

The dependent variables in this study were measured as follows:

(i) Body weight of Dams
Each dam was weighed with the weighing balance from the first day of the experiment and this continued all through gestation until parturition and until the pups are weaned. The daily weight of the dams constitutes body weight.
(ii) Weight of Pups
The weight of all the litters from each dam taken daily divided by the number of pups constitute an average weight for each pup.
(iii) Litter size

This is the number of pups littered by each pregnant rat counted at the nearest experimental observation on time after delivery.

(iv) Infant Mortality
This was obtained by counting the number of pups found dead between the days of parturition and weaning.

(v) Malformation of pups
The malformation is the presence of an anomaly in the pups in the areas of limb development absence of neck or tail. The pups were inspected daily from the date of parturition till weaning for any of such anomaly and recorded.

(vi) Exploratory learning behaviour
The pups were trained to run the maze from the go box to the radial arm of the maze and the time taken to move from go box to the radial arm was taken as a measure of the exploratory learning behaviour on the T-maze.

RESULTS

The data obtained for the study were analyzed using one-way analysis of variance (ANOVA) and t-test for independent measures as appropriate for the various hypotheses tested.

Hypothesis One, which stated that dams in the control group would significantly gain more weight than dams in the low and high cyanide treatment groups was analyzed using one-way analysis of variance. The result is presented in table 1

Table 1: Summary of one-way ANOVA showing the effect of low and high doses of cyanide and saline on body weight of dams

Source of variable		SS	DF	MS	F	P
Body weight of dams	Between groups	5198.47	3	1732.83	21.18	<.001
	Within-group	1308.80	16	81.80		
	Total	**6507.78**	**19**			

Table 1 shows that there is a significant effect of cyanide on the body weight of dams. Dams in the control group significantly gained more weight than dams in the high and low cyanide treatment groups ($F_{(3.16)}$ = 21.18, P<.01).

A post-hoc test was conducted to determine where the differences in body weight existed among the groups. This is presented in Table 2

Table 2: Summary table of Scheffe multiple comparisons test showing where the differences between groups lie in the effect of the Independent variables on the dependent variable

	Variables	1	2	3	4	N	$\bar{X}$	S.D
1	0.1mg cyanide		* * 15.51	* * -35.44	* * 40.31	5	208.73	12.81
2	0.4mg cyanide			* * -19.93	* * 24.80	5	193.22	2.78
3	Control with saline				4.86	5	226.6	12.08
4	Control with no treatment					5	233.5	3.40

* The mean difference is significant at the 0.5 level.

Result of post hoc analysis presented in table 2 above revealed that cyanide had a significant effect on body weight of dams. Dams in the low dose cyanide group significantly gained more weight ($\bar{x}$ = 208.73) than those in the high dose cyanide group. ($\bar{x}$ = 193.22). Also, Dams in the

control group with saline showed significantly higher weight gain ($\bar{x}$ = 226.6) than the cyanide group followed in that order with Dams in the control group without treatment which showed higher body weight ($\bar{x}$ = 233.5) than Dams in the other treatment groups.

Hypothesis Two which stated that malformation of pups, infant mortality, stillbirth, low litter size, and low birth weight would be significantly higher in the high cyanide treatment group than the low cyanide treatment group and control group was analyzed using one-way analysis of variance (ONE-WAY ANOVA). The result is presented in Table 3.

Table 3: Summary of one-way ANOVA showing the effect of low and high doses of cyanide and saline administered to pregnant dams on pup malformation, litter mortality, litter size, and birth weight on offspring of Dams

Source of variable		SS	DF	MS	F	P
Litter size	Between-group Within-group	15.40 38.40	3 16	5.13 2.40	2.14	>.05
	Total	**53.80**	**19**			
Litter weight	Between-group Within-group	641.03 1615.05	3 15	213.68 107.67	1.99	>.05
	Total	**2256.07**	**18**			
Litter mortality	Between-group Within-group	36.96 2.75	2 4	18.48 0.69	26.88	<.05
	Total	**39.71**	**6**			
	Between-group Within-group	1384.93 32096.34	3 13	4614.31 2468.95	1.86	>.05
	Total	**45939.27**	**16**			

Table 3 shows that there was no significant difference between the groups on litter size ($F_{(3.16)}$ = 2.14, P>.05), litter weight ($F_{(3.15)}$ = 1.99, P>.05) and weight of pups ($F_{(3.13)}$ = 1.86, P>.05, while mortality was shown to be significant ($F_{(2.4)}$ = 26.88 , P<.05). To determine the extent to which the groups varied on litter mortality a table of mean scores on all the groups on infant mortality is presented in table 4.

Table 4: Summary of mean scores on infant mortality as determined by the effect of low and high doses of cyanide and control group

Treatment Group	N	$\bar{X}$	SD
0.1mg cyanide	15	3.2	0.95
0.4mg cyanide	12	8.0	.00
Control with saline	35	00	-
Control with no treatment	[illegible]	00	-

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Result in Table 4 showed that there was more infant mortality in the high dose of cyanide group ($\bar{x}$ = 8.0) than the low dose cyanide group ($\bar{x}$ = 3.2) and no cases of infant mortality was recorded in the control groups.

Hypothesis Three, which stated that weanlings prenatally exposed to saline and no treatment would significantly perform better on the exploratory learning task than weanlings prenatally exposed to high and low doses of cyanide, was analyzed using one-way analysis of variance (ANOVA). The result is presented in Table 5

Table 5: Summary of one-way ANOVA showing the effect of low and high doses of cyanide and saline on Exploratory Learning Behaviour of offspring of Dams

Source of variable		SS	DF	MS	F	P
	Between Group	121831.62	3	40610.54	14.35	<.001
	Within Group	101910.27	36	2830.83		
	Total	**223741.89**	**39**			

Table 5 shows that there is a significant difference in exploratory learning behaviour of the offspring of dams in the high and low dose of the cyanide treatment group and the control group. ($F_{(3,\ 36)}$ + 14.35, P = <.001). A post hoc test was conducted to determine where the difference in exploratory learning behaviour existed among the groups. This is presented in Table 6.

Table 6: Summary table of Scheffe multiple comparison test showing differences in mean values between offspring of Dams in the low and high dose cyanide treatment groups and control group on the T-maze learning

	Variable	1	2	3	4	N	$\bar{X}$	SD
1	0.1mg cyanide	-	*64.89	*126.53	*13843	10	147.54	55.24
2	0.4mg cyanide	-	-	*61.64	*73.54	10	212.43	85.52
3	Control with saline	-	-	-	11.90	10	85.9	24.66
4	Control without treatment	-	-	-	-	10	74.00	18.67

***** The mean difference is significant at the .05 level.

The result of the post hoc analysis presented in Table 6 above shows that cyanide had a significant effect on T-maze learning of the offspring of dams. Offspring of dams in the control groups without treatment ($\bar{x}$ = 74.00) and the control group with saline ($\bar{x}$ = 85.9) learned better than offspring of dams in the high ($\bar{x}$ = 212.43) and low dose (147.54) cyanide groups. Offspring of dams in the low cyanide group showed better learning than offspring of Dams in the high cyanide does. ($\bar{x}$ = 212.43).

Hypothesis Four which stated that offspring of dams in the experimental group would significantly show a lower growth rate than offspring of dams in the control group was analyzed using the t-test for independent groups. The result is presented in Table 7.

Table 7: T-test summary table showing differences between offspring of Dams in the experimental and control group on the developmental growth

Variable	Treatment Group	N	$\bar{X}$	SD	DF	t	P
Developmental growth	Experimental group	27	181.24	15.24	15	.56	>.005
	Control	73	196.39	84.56			
Total		**100**					

Result in Table 7 shows that there was no significant difference in the developmental growth rate of offspring of Dams in the experimental group (low and high dose cyanide) and control group (saline treatment and no treatment group), t = 0.58, df = 15; P > .05. thereby rejecting the predictions of hypothesis four.

DISCUSSION

The study with the primary aim of investigating the toxicity impact of cyanide on pregnant mothers and their offspring were carried out using laboratory animals. Four hypotheses were tested to investigate the various psychopharmacological effects of cyanide on pregnant rats and their offspring.

The prediction that dams in the control group would significantly gain more weight than dams in the low and high cyanide treatment groups which clearly defined the effect of cyanide on the body weight of dams in the different treatment groups was supported by the results of the one-way ANOVA adopted for the analysis ($F_{(3.16)} = 21.18$, P<.01).

The significant body weight effect is consistent with the findings of Sousa *et al* (2002), who reported decreased body weight gain as one of the effects of exposure to copper cyanide, potassium cyanide, and silver cyanide. The researchers reported that body weight gain was significantly reduced in male rats that ingested 3.6mg cyanide/kg/day as potassium cyanide in drinking water for 15 days. They reported also that the effect was significant as early as the first week of treatment. This is also consistent with observation seen among children population subsisting mainly on cassava because of its poor protein and cyanogenic content (Banea-Mayambu et al. 2000; Nunn et al. 2011). These findings are consistent with the results of this study.

The result of a post hoc test presented in Table 4.2 revealed that dams in the high dose cyanide group significantly had decreased weight gain (x=193) than dams in the low dose cyanide group ($\bar{x}$ = 208.73) and control groups of saline ($\bar{x}$ = 226.6) and no treatment ($\bar{x}$ = 233.5). Similar findings were reported by Singh (1981), Okolie and Osagie (1992); Frakes *et al* (1986) and Tewe and Maner (1981). Most of the reported cases of body weight loss were investigated using male rats (Sousa *et al* 2002; NTP 1993; Gehart 1986; Gehart, 1987) and only a few reported cases of research using pregnant dams (Frakes *et al* 1986).

However, cases of reduced body weight were reported in a study with male goats and not with lactating female goats (Soto-Blanco *et al* 2001a) although the findings cannot be extrapolated to humans because of differences in gastrointestinal physiology in the ruminant stomach, which render the species an inappropriate model to extrapolation for monogastric species such as humans. Cyanide is highly toxic, especially at high doses. At lethal doses, most animals die before observations are made (ATSDR 1997). Loss of body weight is highly dose dependent.

(WHO 2004; Tewe and Maner, 1981a) consistent with the findings of this research. Cassava is a staple food for about 450-500 million people in the world, mostly in the tropical countries, is consumed in Nigeria especially by the low-income groups who lack the resources to supplement their diets with sulphur enriched diets for the detoxification of cyanide and to increase the palatability of the meals (WHO 2004). The most plausible explanation for the observed effect of cyanide on weight loss is the possible decreased food and water consumption by the dams due to decreased palatability of meals. According to WHO (2004), oral cyanide ingestion reduces the palatability of meals consumed by the subjects. Similar findings were reported by Leuschner *et al* (1989). Pregnancy being a critical condition that affects the mother may even contribute to loss of appetite, thereby leading to weight loss.

The effect of cyanide on the offspring of dams exposed prenatally to cyanide was partially supported by the results of the study. Fetal developmental abnormalities like malformations, infant mortality, stillbirth, low Litter size, and low birth weight were postulated by the hypothesis to be significantly high at the high dose of exposure employed in the study. However, the results showed that there was no significant difference on all variables except infant mortality, which was observed to be significant ($F_{(2.4)}$ = 26.88, P<.05) and more pronounced at the high dose of 0.4mg cyanide/kg body weight ($\bar{x}$ = 8.0) and less at 0.1mg/kg body weight ($\bar{x}$ = 3.2) and there was no infant mortality in the control groups, as shown in Table 4.4. These findings are consistent with the findings of Singh (1981), who observed developmental abnormalities in 28% of fetuses of rats exposed to cassava meals during gestation. Whillhite (1982) confirmed that fetal abnormalities can only be observed at doses which are toxic to the mothers. In other words, the mothers would have all died before the offspring were delivered and as such the abnormalities might not be observed. However, post mortem investigations made on fetuses of dams exposed to cyanide during gestation has shown that cyanide causes fetal abnormalities like limb defects, micro-encephally, rib abnormalities, reduced fetal weight, delayed ossification, and congenital hypothyroidism (Frakes et al 1986; Singh, 1981; Willhite, 1982; NRC, 1993).

However, Tewe and Maner (1981), in an experiment using cassava diet liberating 21mg hydrogen cyanide on pregnant Wister rats reported no cases of birth defects on the offspring. The same observations were made using Yorkshire pigs, who were treated with potassium cyanide (Tewe and Maner 1981b). These results are consistent with the findings of this research.

It must be noted that the results of research findings showing no cases of birth defect and not an indication that prenatal exposure to cyanide does not bring about birth defects on the offspring. It only suggests that the prevalence of birth defects from cyanide exposure is low, and can only be observed from mothers who survive acute exposures to cyanide. According to Singh (1981), 28% of fetuses exposed to cyanide showed signs of abnormalities, which may be reduced in some other circumstances especially in conditions of improved diets (ATSDR, 2001c).

The incidence of infant mortality, which was significantly evident in the cyanide group, confirms the high susceptibility of litters to cyanide exposure (ATSDR, 1997). It might be assumed that since the dams survived the chronic administration of cyanide, their offspring will also survive. However, according to Guzelian (1992) infants are more susceptible than adults to health effects of hazardous chemicals, and vulnerability depends on the developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during these critical periods. Damage may be lethal and may not be evident until a later stage of development

(NRC 1993; Komori *et al* 1990; Leader and Kearns 1997; Viera *et al* 1996). The effect of cyanide on infants appears to be more pronounced than those of similarly exposed adults (ATSDR, 1997). This is consistent with research findings on cyanide's inhibition of mitochondrial respiration in all cells and reported cases of headache, coma, abnormal respiration, hypotension and eventual death in infants (Lasch and Elshawa, 1981; ATSDR, 1997; WHO 2004). Cyanide inhibits respiration in cells and developing infants breathe more air per kilogram of body weight than adults, which accounts for high susceptibility of the infants to cyanide exposure. Potassium cyanide, sodium cyanide, and other cyanogenic compounds exert their acute toxic effect by complexing with the ferric ion-atom in the metalloenzymes, resulting in the histotoxic anoxia through inhibition of cytochrome C oxidase (ATSDR, 1997).

According to research reports, the central nervous system is the primary target for cyanide toxicity in humans and animals, which may be stimulated by acute cyanide (Singh *et al* 1989; ATSDR 1997; WHO 2004). The effects of cyanide on the central nervous system are probably due to rapid biochemical changes in the brain such as ion flux neurotransmitter release and possible peroxide formation (ATSDR 1997; Kanthasamy *et al*; 1994; Kanthasamy *et al* 1991; Johnson and Isom, 1985) These biochemical changes in the brain usually bring about the manifestation of certain behaviours that are consistent with the part of the brain with the chemical insult. In line with this, the third hypothesis of this study postulated that weanlings prenatally exposed to saline and no treatment would perform better on the learning task than weanlings prenatally exposed to high and low doses of cyanide. The results obtained from the study confirmed the hypothesis ($F_{(3, 36)} = 14.35$, $P<.001$). A comparison of means also showed that offspring of Dams in the control group without treatment was better in the learning task ($\bar{x}=74.00$) than those in the low cyanide group ($\bar{x}=147.54$) and high cyanide group ($\bar{x}=212.43$) in that order.

These findings are consistent with the results obtained by Jackson (1988), who observed behavioural changes in pigs after oral exposure to potassium cyanide. Though the observed behavioural changes were not defined by the researcher, cyanide targets the central nervous system which controls all aspects of behaviour in humans and animals. Other research findings have reported some neurological effects (memory loss and a parkinsonian-type syndrome) as delayed effects following accidental acute ingestion of soluble cyanide compounds (Chin and Calderon 2000; Rachinger *et al* 2002; Rosenow *et al* 1995). Also in consonance with the findings of this research that cyanide has an effect on learning, which is related to memory, Chin and Calderon (2000) reported the case of a female who developed difficulties with short term memory 5 months after ingesting an unknown amount of an unspecified cyanide compound.

Though there is dearth of research on acute cyanide exposure on learning behaviour and memory on humans and animals, explanations on observed effects in this study can be proffered from studies on the target organ toxicity of cyanide. However, more work is required in this area to carry out further investigations on the anatomical and biochemical tests after the acute experiment and behavioural observations carried out in this study. Lesion studies on the brains and spinal cords of animals exposed to cyanide show enough evidence that cyanide produces excitotoxic responses in select brain areas (Patel *et al* 1991; 1992; 1993). Inhalation and oral studies in animals have shown that acute or chronic cyanide exposure leads to encephalopathy in both white and gray matter. In particular, the damage has been observed in regions of the brain such as deep ce 103 corpora striata, pallidum and substantia nigra (Gunaskar *et al* 2000). Research findings have shown that damage to the hippocampus may lead to an inability to learn some behaviour. According to O'Keefe and Nadal (1978), one of the learning systems referred to as the taxon is reliant upon the hippocampus and supports

stimulus-response learning or habit learning. Lesion studies by Jarrad (1983) show that rats with hippocampal lesions are impaired in learning the radial arm of a maze. Other studies show that hippocampal lesioned rats are impaired in swimming to a hidden platform of a T-maze (Morris, 2001; Pearce *et al*; 1998).

Research evidence shows that the activities of the hippocampal neurons in both animal and human species reflect information about the spatial organization of an animals environment. Current research has shown that the hippocampus in rats may contribute to more than one type of spatial learning navigation. Path integration is a form of spatial learning navigation in which an animal integrates self-movement cues (Vestibular information) to locate its present position or return to a starting location. This ability has been shown in different species (Mittelstaedt and Mittelstaedt 2001).

The hippocampus has also been implicated as the region of the brain responsible for the control of short-term memory. Studies on patients suffering from Amnesia (forgetfulness) using the Magnetic Resonance Imaging (MRI) have shown that damage to the hippocampus and the neighbouring structures was responsible for the condition suffered by the patients (Corkin *et al*, 1997; Hilts, 1995). In an experimental study, using animals, Kesner *et al*, (1993) designed a series of tasks, each of which would make animals focus especially on a particular attribute of experience, to test only working short-term memory. For spatial recognition tasks, Kesner *et al* (1993) used the eight arm radial maze and exposed the rats to pre-training exercises with food at the end of each arm. Different groups of animals were tested after having received a sham lesion in the hippocampus, the caudate nucleus, or the extrastriate cortex. The results of the experiment showed that only the animals with hippocampal lesions made more mistakes than the controls (animals with sham lesions) on the spatial task. A similar study by McDonald and White (1993) confirms that hippocampus is implicated in the acquisition of information about relationships among stimuli and events. Damage to the hippocampus by cyanide exposure which research evidence has shown to be dose-dependent (Rachinger *et al* 2002), is assumed to be the only possible explanation for the observed performance of the cyanide group of rats on the T-maze task as compared to the control group which is consistent with the results obtained from the research results explained above (Corkin *et al* 1997; Hilts, 1995;Kesner *et al* 1993; McDonald and White 1993).

It has been reported that survivors of cyanide poisoning similar to conditions of continued exposure of systematic doses of cyanide may develop parkinsonian-like signs with lesions in the substantia nigra which is a dopaminergic centre. This has been confirmed with studies using Magnetic Resonance Imaging (MRI) (Chin and Calderon 2000, Rachinger *et al* 2002; Rosenow *et al* 1995). According to Prabhakaran *et al* (2002), sub-acute exposure to cyanide produces necrotic lesions within the striatum and basal ganglia, the brain areas that are involved with the movement. This leads to the development of Parkinson-like syndrome in animals similar to that reported in human toxicity following cyanide exposure (Prabhakaran *et al* 2002). The manifestation of spontaneous actions great difficulty in all motor efforts, slow movement and tremors of the hand as in Parkinson's disease could contribute to the inability of the rats in the cyanide group to effectively perform the learning task on the T-maze. The slow rate at which rats in the high dose cyanide group completed the maze task ($\bar{x}$ = 212.43) compared to low dose cyanide group ($\bar{x}$ = 147.54), control group with (saline) ($\bar{x}$ = 85.9) and control group (no treatment) ($\bar{x}$ = 74.00) is an indication that the condition described above may be a contributory factor to the results obtained in this study.

Another plausible explanation for the inability of offspring in the cyanide group to learn better on the T-maze task is the depletion of iodine as a result of exposure to cyanide. Thiocyanate, the major detoxification product of cyanide prevents the uptake of iodine and acts as a goitrogenic agent, (USEPA, 1990; ATSDR, 1993). Iodine deficiency in infants has been implicated as responsible for several neuropsychological impairments; in the fetus, one of such impairments is retardation in fetal brain development which in turn may impair other neuropsychological processes, (Fenzi *et al* 1990; Vermiglio *et al* 1990). Thyroid hormones are the only substances produced in the body that contain iodine and their synthesize manufacture is critically dependent on the supply of iodine. The thyroid hormones influence growth which is very evident when thyroid deficiency starts early in life due to unavailability of iodine. Besides stunted body growth, there is marked a reduction in brain size and cellular structure. (Delange *et al* 1994).

The results did not support the predictions that offspring of dams in the experimental group will significantly show lower growth rate than offspring of dams in the control group ($t = 0.58$, $df = 15$; $P>.05$). Though the offspring of Dams in the control group gained more weight during the lactation period ($\bar{x} = 196.39$) than those in the experimental group ($\bar{x} = 181.24$), however, it was not significant enough to make a difference. Some research evidence from cassava eating populations show that hypothyroidism leading to cretinism may occur from gestational exposure to cyanide (Soto-Blanco and Gormiak 2003) and from lactating ewes that cyanide can be transferred to in the milk of exposed goats (Soto-Blanco and Gormiak, 2003). The probable reason for the offspring in the cyanide group to develop almost as well as the control group is the availability of diets that are enriched with enough protein to detoxify cyanide. Though the effects manifested in some of the offspring, it was not significant to make a difference. The rats were fed with mouse cubes which contain balanced diets supplying all the nutrients needed to take care of the lactating pup.

CONCLUSION

The main conclusions based on the findings of this study are summarized as follows;

1. There is a strong relationship between cyanide ingestion and overall wellbeing of pregnant dams;
2. Cyanide ingestion during pregnancy has some far-reaching effects on pregnant dams and their litters;
3. Observed toxic effects of cyanide on pregnant dams or the litters depend on the level of exposure, which has a direct relationship with the dose;
4. Cyanide is highly toxic like any other poison in food or drugs but can be tolerated at very low doses, and is certainly highly toxic at doses above the lethal dose;
5. Prenatal exposure to cyanide increases the incidence of litter mortality due to susceptibility of litters to the toxic effects of cyanide;
6. There is no significant effect of cyanide ingestion by the dams on the weight of litters;
7. There is no significant effect of cyanide exposure of dams on the litter size (i.e. number of pups littered by the dams.)
8. Cyanide ingestion by dams does not significantly affect the development weight of the litters;
9. Cyanide ingestion by the dams significantly affects the exploratory learning behaviour of the litters.

RECOMMENDATIONS

The following recommendations are made based on the results obtained from this study and the discussions:

1. It is recommended that women, especially pregnant women, should be adequately catered for during pregnancy in terms of the food they consume;
2. Pregnant women should avoid consuming food items that may be injurious to the fetus and eventually the offspring. (In the light of this, cassava products, which are the major source of cyanide exposure in Nigeria, should be reduced);
3. The general public and pregnant women, in particular, should be adequately provided with enough protein sources to facilitate the process of detoxification of cyanide in the blood;
4. Since cassava is a major staple food in Nigeria, it may be difficult to ask people to abstain from cassava consumption. It is therefore recommended that the government at all levels should provide the farmers with a strain of cassava that is low in cyanogenic glycoside content;
5. Intensive campaign and education should be mounted by the department of agriculture in all rural communities with the aim of educating the people on the best methods of processing cassava, to eliminate or reduce the cyanide content to a non-toxic level;
6. Since most of the reported cases of cyanide poisoning through cassava consumption are from the low-income groups of the society, it is recommended that government, societies, and individuals should help to improve the quality of life of the less privileged members of the society in order to reduce the impact of the consumption of inadequately processed cassava due to poverty.

REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR) 2004. *Toxicological Profile for cyanide.* Atlanta, GA: US Department of Health and Human Services, Public Health.

ATSDR (1991) case studies in environmental medicine. Atlanta GA. US Department of Health and Human services, public health services, Agency for Toxic Substances and Disease Registry.

ATSDR (1997) Toxicological Profile of cyanide. Atlanta, GA. US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry (ATSDR)

ATSDR (2001) Draft guidance manual for the assessment of joint toxic action of chemical mixtures. Atlanta, G.A: Agency for Toxic Substances and Disease Registry.

Ballantyne, B. (1983). Artifacts in the definition of toxicity by cyanide and cyanogens. *Fundamentals of Applied Toxicology,* 3:400.

Banea, M., Poulter, N., & Rosling, H. (1992). Shortcuts in cassava processing and risk of dietary cyanide exposure in Zaire. *Food and Nutrition Bulletin14*: 137-143.

Banea-Mayambu, J.P; Tyleskar, T; Gitebo, N; Matadi, N; Gebre-Medhin, M; & Rosling, H. (1997). Geographical and seasonal association between linamarin and cyanide exposure from cassava and upper motor neuron disease 'Konzo' in former Zaire. *Tropical Medicine and International Health*, 2:1143-1151

Banea-Mayambu, J.P; Tylleskar, T; Tylleskar, K; Gebre-Medhin, M; & Rosling, H (2002). Dietary cyanide from insufficiently processed cassava and growth retardation in children in the Democratic Republic of Congo (formerly Zaire) *Annals of Tropical Paediatrics*, 20:34-40.

Chin, R.G. & Calderon Y. (2000) Acute cyanide poisoning: A case report. *Journal of Emergency medicine*, 18: 441-445.

Corkin, S., Amaral, D.G., Gonzalez, R.G. Johnson, K.A., & Hyman B.T.C. (1997). H.M.'s Medical temporal lobe lesion: Findings from Magnetic Resonance Imaging. *Journal of Neuroscience,* 17; 3964-3979.

Delange, F. Ekpechi, L., & Rosling, H. (1994). Cassava cyanogenesis and iodine deficiency disorder. *ACTA Horticulture* 375: 289-293

Ernest, M; Cardoso, A.P; Nicala, D, Mirione, E; Massaza, F; Cliff J, Haque, M.R & Bradbury, J.H. (2002). Persistent 'Konzo' and cyanogen toxicity from cassava in Northern Mozambique *Acta Tropical,* 82:357-362.

Fenzi, G.F., Giusti, L.F., Aghini Lambardi, F., Bartalena, L., /Marococci, C., Santini, F., Bargagna, G., Falciglia, G., Monteleone, M., Marcheschi, M. &Pinchera, A. (1990) Neuropsychological Assessment in school children from an area of moderate iodine deficiency. *Journal of Endocrine Investigation*, 13: 427-431

Frakes, R.A. Sharma, R.P. & Willhite C.C. (1986) Comparative metabolism of linamarin and amygdalin in hamsters. Food and chemical *Toxicology*,24: 417-420.

Gerhart, J.M. (1986) Ninety-day oral toxicity study of copper (CUCN) in sprague-Dawley rats. Prepared for the Dynamic Corporation, Rockville, M.D., by IIT Research Institute, Chicago, IL (IITRI Project N0. L06183, study N0. 3) cited in ATSDR 1997. Gerhart, J.M. (1987). Ninety-day oral toxicity study of potassium silver cyanide {Kag (CN_2} in sprague-Dawley rats. Prepared for the Dynamac Corporation, Rockville, M.D, by IIT Research Institute, Chicago. IL (IITRI Project N0 L06183, study N0 4) cited in ATSDR, 1997.

Guzelian, P.S., Henry, C.J. & Olin, S.S. (eds) (1992). *Similarities and differences between children and adults: Implication for risk assessment.* Washington D.C. International Life Science Institute Press.

Hilts, P.J. (1995) *Memory's ghost. The strange tale of Mr. M and the nature of memory*, New York: Simon and Schuster.

Jackson, L.C. (1988). Behavioural effects of chronic sublethal dietary cyanide in an animal model. Implications for humans consuming cassava (Maninotesculenta) *Human Biology* 60: 597-614.

JECFA (1993) Cyanogenic glycosides in *Toxicological evaluation of certain food additives and naturally occurring toxicants*. Geneva, World Health Organization, 39th meeting in the joint FAO/WHO Expert Committee on Food additives (WHO Food Additives Series 30)

Kanthassamy, A.G., Isom, G.E. & Borowitz, J.L. (1995). Role of intracellular Ca^{2f} in catecholamine release and lethality in PC12 cells. *Toxicology Letters,* 81:151-157.

Kanthassamy, A.G., Sun, P.W., Borowitz J.L; Kane, M.D. Gunaskar, P.G. & Isom, G.E. (1996). Cyanide-induced neurotoxicity involves nitric oxide and reactive oxygen species generation after N-methyl-D- aspartate receptor activation. *Journal of Pharmacology an Experimental Therapy,* 277: 150-155

Kanthasamy, A.G; Maduh, E.U., & Peoples, R.W. (1991). calcium mediation of cyanide-induced catecholamine release: Implications for neurotoxicity. *Toxicology and Applied Pharmacology*, 110:275-282.

Kanthasamy, A.G., Borowitz, J.L., & Parlakovic, G. (1994). Dopaminergic neurotoxicity of cyanide: neurochemical, histological and behavioural characterization. *Toxicology and applied pharamacology,* 126(1): 156-163.

Kesner, R.P; Bolland B.L; and Dake, M. (1993). Memory for spatial locations, motor responses, and objects: triple dissociation among the hippocampus, caudate nucleus and extrastriate visual cortex. *Experimental Brain Research,* 93; 462-470.

Komori, M., Nishio, K., & Kitada, M. (1990). Fetus – specific expression of a form of cytochrome p-450 in human livers. *Biochemistry* 29: 4430-4433

Lasch, E.E., & Elshawa, R. (1981) Multiple cases of cyanide poisoning by apricot Kernels in children from Gaza. *Pediatrics*, 68: 5-7.

Leeder, J.S. & Kearns, G.L. (1997). Pharmacogenetics in the pediatrics: Implication for practice. *Pediatric Clinic of North America,* 44 (1): 55-77.

Leuschner, J., Winkler, A., & Leuschner, F. (1991) Toxicokinetic aspects of chronic cyanide exposure in the rat. *Toxicology Letters* 57: 195-201

Leuschner, F. Neuman, B.W., Otto, H., & Moller, E. (1989). 13-week toxicity study of potassium cyanide administered to Sprague-Dawley rats in the drinking water. Unpublished study, Laboratory of Pharmacology and Toxicology, July (cited in JECFA, 1993) *and oncology,*5: 151-163 Litovitz, T.L., Martin, T.G., Schmitz, B., (1987). Annual report of the American Association of poison control centers National Data Collection System. *American Journal of Emergent Medicine* 5: 405.

McDonald, R.J. & White, N.M. (1993) A triple dissociation of memory systems: Hippocampus, amygdala and dorsal striatum. *Behavioural Neuroscience* 107, 3-22.

Mittelstaedt, M.L. & Mittelstaedt, H. (2001) ideothetic navigation in humans: estimation of path length. *Experimental Brain Research*, 139: 318-332.

Nartey, F. (1981). Cyanogenesis in tropical feeds and foodstuffs: In Vennesland, B. et al. *Cyanide in Biology.* New York Academy Press.

Narty, F. (1980). Toxicological aspects of cyanogenesis in tropical foods. In: Smith R.L. and Bababunmi E.R., eds. *Toxicology in the Tropics*. London, Taylor and Francis, 53-73. (cited in JECFA, 1993)

NRC: (1993). *Pesticides in the diets of infants and children.* Washington D.C: National Academy Press. National Research Council.

NTP (1993). Sodium cyanide administered in drinking water to F344/N rats and B6C3F, mice. Research Triangle Park, NC, National Institute of Health, Toxicology Program (Toxicology Report Series No 37: NH Publication 94 – 3386).

Nunn, J.A; Graydon, F.J; Polkey, C.E, & Morris, R.G. (1999). Differential spatial memory impairment after right temporal lobectomy demonstrated using temporal titration. *Brain*, 122:47-59.Nweke, F., Spencer, D., and Lynam, J (2002). *The Cassava Transformation*: Africa's best-kept Secret. *Mich Press, East Lansing, USA.*

O'keefe, J, & Nadal, L. (1978) *The Hippocampus as a Cognitive Map.* Oxford University Press.

Okolie, N.P, & Ironya, C.U., (2003) Some histological and Biochemical evidence for mitigation of cyanide-induced tissue lesions by antioxidant vitamin administration in rabbits. *Food and Chemical Toxicology* 41; 463-469

Okolie, N.P & Osagie, A.U.(2000) Differential effects of chronic cyanide intoxication on heart, lung and pancreatic tissues. *Food and Chemical Toxicology* 38; 343-548

Okolie, N.P & Osagie, A.U. (1999). Liver and kidney lesions and associated enzyme induced rabbits by chronic cyanide exposure. *Food Chemistry and Toxicology* 37(7): 745-750.

Padmaja A. (1996). The culprit in cassava toxicity: cyanogens or low protein. *Consultative Group on International Agricultural Research (CGIAR News).* 3(3)

Padmaja, A (1995) Cyanide detoxification in cassava for food and feed use. pathogenesis of Konzo. Neural cell damage. *Lancet* 339: 208-211

Patel, M.N. Ardelt. B.K., Yim, G.K. & Isom G.E. (1991). Cyanide induces ca^{2++} - dependent and independent release of glutamate from mouse brain slices. *Neuroscience Letters*. 131: 42-44

Patel, M.N. Peoples, R.W. Yim, G.K.W., & Isom, G.E. (1992) Enhancement of NMDA – mediated responses by cyanide. *Neurochemical Research*, 19: 1319-1323

Patel, M.N., Yim, G.K.W., & Isom, G.E (1993) N – methyl-D-aspartate receptors mediate cyanide induced cytotoxicity in hippocampal cultures. *Neurotoxicology* 14: 35-40

Pearce, J.M; Roberts, A.D; & Good, M, (1998). Hippocampal lesions disrupt navigation based on cognitive maps but not heading vectors. *Nature, 396*, 75-77

Prabhakaran, K., Li, L., Borowitz, J.L., & ISom, G.E. (2004) Caspase inhibition switches the mode of cell death induced by cyanide by enhancing reactive oxygen species generation and PARP-1 activation. *Toxicology and Applied Pharmacology. 195:* 194-202.

Prabhakaran, K., Li. L; Borowitz, J.L., & Isom, G.E. (2002). Cyanide induces different odes of death in the cortical and mesencephalic cells. *Journal of Pharmacology and Experimental Therapy* 302, 510-519.

Rachinger, J., Felner, F.A., & Stieglbauer K. (2002) MR changes after acute cyanide intoxication. *American Journal of Neuroradiology*, 23: 1398-1401

Rosenow, F., Herholz, K., Lanfermann, H., Weathen, G., Ebner, R. Kessler, J., Ghaemi, M & Heiss, W.D. (1995). Neurological sequel of cyanide intoxication – the pattern of clinical magnetic resonance imaging and positron emission tomography findings. *Annals of Neurology*, 38: 825-28.

Singh, J.D. (1981). The teratogenic effects of dietary cassava on the pregnant albino rat: Preliminary report. *Teratology*, 24: 289-291

Singh, M., Coles, N., & Lewis, P. (1989). The metabolic effects of fatal cyanide poisoning. *Postgraduate medical journal65:* 923-925.

Soto-Blanco, B., & Gorniak, S.L (2003). Effect of long term low dose cyanide administration to rats. *Ecotoxicology and Environmental safety*, 53:37-41.

Soto-Blanco, B., Maiorka, P.C. & Gorniak, S.L. (2002). A neuropathological study of long term cyanide administration to goats. *Food and Chemical Toxicology*, 40: 1693-1698

Sousa, A.B., Soto-Blanco, B., Guerra, J.L., Kimura, E.T., & Gorniak, S.L. (2003). Does prolonged oral exposure to cyanide promote hepatotoxicity and nephrotoxicity? *Toxicology,* 53: 37-41.

Sreeja, V; Nagahara, N; Li, Q, & Minamic, M (2003). Neural aspects in the pathogenesis of Konzo. Neural cell damage. *Lancet* 339: 208-211

Tewe, O. (1983). Thyroid cassava toxicity in animals. In Delange, F. Ahaluwahq.R. (eds). *Cassava toxicity and thyroid and public health issues.* IDRC, Ottawa Canada.

Tewe, O.O. & Maner, J.H. (1981a) Long-term and carry-over effect of dietary inorganic cyanide (KCN) in the life cycle performance and metabolism of rats. *Toxicology and Applied Pharmacology*, 58:1-12

Tewe, O.O. & Maner, J.H. (1981b). Performance and pathophysiological changes in pregnant pigs fed cassava diets containing different levels of cyanide. *Research in Veterinary Science,* 30: 147-151

USEPA (1992). Drinking water criteria document or cyanide Environmental criteria and Assessment Branch.

USEPA (1996). Drinking water regulations and Health Advisories.

USEPA (2004) *Cyanide Compounds Hazard Summary* Atlanta GA.

USEPA (2003) Classes of underground injection wells. Cyanide clarification. Labcert Bulletin. US Environmental Protection Agency.

Vermiglio, F., Sidoti, M., Finochiaro, M.D., Battiato, S., Lo Presti, V.P. Benvenga, S; & Trimarchi, F.D., (1990). Defective neuromotor and cognitive ability in iodine-deficient school children of an endemic goiter region in Sicily. *Journal of Clinical Endocrinology and Metabolism*, 70: 379-384

Viera, L., Sonnier, M; & Cresteil, T. (1996) developmental expression of CYP2EI in the human liver. Hypermethylation control of gene expression during the neonatal period. *European Journal of Biochemistry* 238: 476-483

W.H.O. (1996) (World Health Organization) Maternal and Newborn Health antenatal care. *Report of a Technical Working group*.

WHO (2004). Hydrogen cyanide and cyanides: Human health aspects (Concise international chemical assessment document, 61) World Health Organization.