



## Damaging Effects Of Phosphide- Powder Residue On Brain, Lung And Heart Histoarchitecture In Rats

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### ABSTRACT

In many parts of the developed world, the sale and use of phosphide is under strict regulations with adequate training of workers being a major prerequisite before its use. In Nigeria this chemical is commonly available and in most cases users are largely untrained, therefore contamination of cowpea with residual phosphide post-fumigation does occur. The aim of this study is to determine the impact of phosphide residue contamination on organs such as brain, lung and heart. Six rats per group were used for this study. The rats in the first and second groups received phosphide residue contaminated and uncontaminated cowpea respectively, while the rats in the third group were fed untreated cowpea and served as control. Tissues (brain, lung, heart) were fixed in 10% formal saline for routine histological techniques and Haematoxylin and Eosin staining technique was used. Feeding of rats with phosphide residue contaminated cowpea resulted in significant damage to all examined tissues. Examination of different sections revealed severe congestion of the meninges (brain); severe congestion at the interalveolar septum with diffuse hemosiderosis (lung); and congestion of coronary vessels (heart) in rats fed cooked phosphide-residue contaminated cowpea. The rats in treated but uncontaminated cowpea and untreated cowpea groups exhibited no visible lesions in all tissue-sections examined. This study has revealed that the erroneous belief among illiterate grain merchants that phosphide residue is harmless should be discarded and proper training of users of this chemical should be encouraged to prevent many of the anomalies associated with cowpea fumigation in Nigeria.

**Keywords:** Brain, lung, heart, phosphide-residue.

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### 1. INTRODUCTION

The discovery of phosphine (PH<sub>3</sub>) in the late 1700s paved way for its subsequent use as a grain fumigant, and it has been in use for the preservation of stored cowpea since 1930s<sup>[1]</sup>. It was not until 1980s that it became the most significant means of controlling pest insects in stored grain and many other stored commodities and for this reason it has been identified as being of great value as per global food security because of the many advantages it has over other fumigants<sup>[2]</sup>. Advantages such as, ease of

application, its effectiveness, lack of residue on stored grains, and its low cost have led to its continual use on nearly all internationally traded grain destined for human consumption as well as for local market.

It is gaseous above -88°C with a density of 1.17 times that of air, which allows it to disperse readily during fumigation. A highly toxic agent to aerobically respiring organisms, but not to anaerobic or metabolically dormant organism; it destroys insect pests in grain, without

affecting grain viability<sup>[3]</sup>. Due to its high degree of toxicity, in the UK aluminum phosphide (ALP) is available in form of tablets or pellets (Phostoxin, Talunex, and Degesch) and the supply is restricted under the Pesticides Act 1998 to qualified users<sup>[4]</sup>. While there is restriction in the use of phosphide in the developed world, in Nigeria and many parts of the developing world, there is ease of availability of this chemical. Exposure of phosphide to moisture results in the release of phosphine gas, which can be absorbed rapidly by inhalation as well as through dermal and gastrointestinal routes.

Dua et al.<sup>[5]</sup> have revealed that the brain and liver are two of the most susceptible tissues to phosphide toxicity, and an earlier study in which phosphide powder residue contaminated cowpea was fed to rats, revealed significant alterations in serum levels of hepatic indices. This present study is designed to investigate if such treatment has any effect on the morphology of the brain cells, so as to determine the extent of tissue damage which can result from poor handling of the grain-fumigation process. A survey of the local market has revealed that extended fumigation period, use of abnormally high phosphide content in relation to quantity of grain and phosphide powder residue contamination of cowpea, are some of the common ways in which cowpea consumers are exposed to toxic effects of this agent, and information abound suggests that consumption of such cowpea have resulted in a myriad of medical complaints. The aim of this study is to identify the impact of phosphide powder residue on brain, lung and heart sections, since the brain cells are highly oxidative cells and unspent phosphide has been recognized to alter the oxidative phosphorylative pathway.

## 2. MATERIALS AND METHODS:

**Animals and animal care:** Eighteen female albino rats (250 g) were obtained from the Animal House of the Department of Veterinary Physiology, University of Ibadan, Nigeria where the study also took place. The animals were kept in cages at ambient temperature of  $23\pm 3^{\circ}\text{C}$  and a 12 h light, 12 h dark cycle and were fed with standard laboratory diet and given water *ad libitum*. The rats were divided equally into 3 groups; the rats in 1<sup>st</sup> and 2<sup>nd</sup> groups were fed with cooked phosphide treated cowpea, but cowpea of the 2<sup>nd</sup> group was deliberately contaminated with phosphide-powder residue. The 3<sup>rd</sup> group served as the control and given untreated cowpea. This study was carried out in conformity with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research; as promulgated and adopted by United States Institutes of Health (1985).

## Treatment of cowpea:

Cowpea was fumigated, using Protex (aluminum phosphide-57% inert ingredients-43%) manufactured by United Phosphorus Ltd, India. Using a ratio of 2 tablets of phosphide per  $\text{m}^3$  of space, cowpea was fumigated at  $29^{\circ}\text{C}$  over a period of 48 hours. At the end of the fumigation process, the grains were separated from the fumigant, and the treated cowpea was divided into two, and one part was deliberately contaminated with phosphide powder residue, i.e. residue of a quarter tablet of Protex was used to contaminate one kilogram of cowpea. The rats were fed their different cowpea type over a period over 8 hours. The study was terminated exactly 24 hours after it commenced.

## Histological study:

The female Wistar rats used for this study were sacrificed by cervical dislocation and their skulls opened using bone forceps to expose the brain of the rats after which the brain quickly dissected and fixed in 10% formal saline for routine histological techniques as well as sections of lung and heart. The tissues obtained were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. A series of sections of 7 microns thick were obtained using a rotatory microtome. These were deparaffinized and stained routinely with Haematoxylin and Eosin. Magnification was at x 400.

## 3. RESULTS AND DISCUSSION:

The results of this study are presented in **Figures 1-3**.

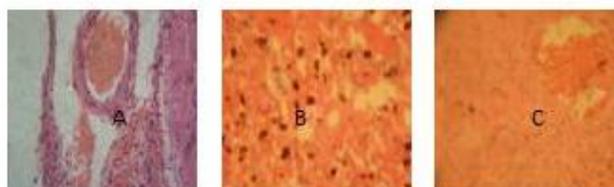


Figure 1: Photomicrographs of A (brain- there is severe congestion of the meninges); B (lung- there is severe congestion at the interalveolar septum, with diffuse hemosiderosis); C (heart- the coronary vessels are congested) of rats fed cooked phosphide-residue contaminated cowpea. Mag X 400



Figure 2: Photomicrographs of D (brain); E (lung); F (heart)- showing no visible lesion of rats fed cooked phosphide treated but uncontaminated cowpea. Mag X 400

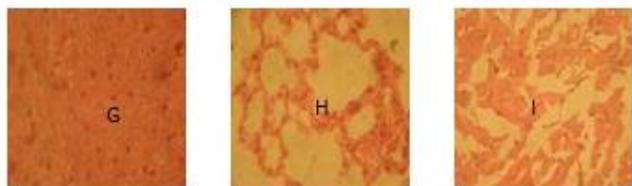


Figure 3: Photomicrographs of G (brain); H (lung); I (heart)- showing no visible lesion of control rats. Mag X 400

Aluminum phosphide (ALP), a highly toxic pesticide, is a mitochondrial toxin that causes death by cardiac and metabolic toxicity. Siddaiah et al.<sup>[6]</sup> have recognized that this chemical's ominous effect is cardiac toxicity, which may range from minor electrocardiographic abnormalities to severe depression of cardiac contractility secondary to toxic myocarditis. This study also confirmed significant abnormalities in the sections of the heart and lung as revealed by histology results. Anger et al.<sup>[7]</sup> have also identified that microscopic examination revealed congestion of all the viscera organs studied and obvious asphyxia lesions in the pulmonary parenchyma.

The effects phosphide residue contaminated cowpea on animals has not been widely explored, since in most cases it is taken for granted that the fumigation process will be carried out according to the manufacturer's instruction but our market survey revealed a number of lapses associated with cowpea fumigation process in Nigeria. Among other anomalies, prolonged fumigation period and phosphide residue contamination of cowpea may be likely causes of many of the complaints commonly associated with cowpea consumption. That such anomaly may result in pathology of the brain may be assumed from the past findings in which, when experimental animals were exposed to phosphine gas, the active derivative of phosphide, abnormal brain presentations were reported.

The work of Dua et al.<sup>[8]</sup> have established that inhibition of cytochrome oxidase causes decline oxygen uptake and decreased ATP synthesis which eventually resulted in cellular energy crisis and may trigger non-oxidative glucose break down, thereby leading to a significant decrease in plasma glucose levels in the ALP treated rats. The decrease in ATP generation which occurs along with hypoglycemia may further intensify the cellular energy deficits. These alterations were more pronounced in brain and liver cells than other tissues according to their study. Moreover, according to them the brain tissue especially in order to meet the sudden increase in the local energy demand, utilizes its stored energy in the form of glycogen breakdown as observed by a decrease in the glycogen levels in the brain cells which was accompanied by a marked increase in the activity of glycogen phosphorylase. That this residue may have a profound effect on the brain can be postulated through another observation made by these workers, they showed that the glycolytic rate was

elevated in brain tissue subsequent to phosphide exposure and this was evident by significant increase in the activities of hexokinase and phosphofructokinase enzymes, while the liver (another susceptible organ) exhibited a decrease in activities of these enzymes in ALP treated rats. The results of their study therefore suggested that ALP caused cellular energy deficit which compromised energy status of the brain as well as induced substantial alterations in glucose homeostasis. However, the activity of glucose-6-phosphate dehydrogenase, an important enzyme in the pento-phosphate pathway was significantly decreased in both tissues. These alterations may be the perquisite for many of the histologic distortion in the cellular architecture of the brain of animals which were fed with phosphide residue contaminated cowpea.

In addition, results of brain histology of treated rats that showed congestion of the meninges probably further suggests that phosphide residue contamination of cowpea may have harmful effect on exposed animals. This is not surprising because a number of pesticides used in agriculture, designed to protect crops against unwanted species, such as weeds, insects, and fungus are known neurotoxic agents. These are pesticides such as organophosphates, carbamates, pyrethroids, ethylenebisdithiocarbamates, and chlorophenoxy which are currently in use, not only in Europe and some other developed parts of the world but in Africa as well. They have been found to cause neurodevelopmental toxicity<sup>[9]</sup>. Moreover, because brain biochemistry of many animal species is identical such pesticides may also be neurotoxic to humans, and there are indications to suggest that the developing brain may be more vulnerable to adverse effects of this pesticide than adult brain. This is because in the course of prenatal life, the human brain must develop from the ectodermal cells of the embryo into a complex organ consisting of billions of precisely located, highly interconnected, and specialised cells. For maximal brain development, neurons must move along precise pathways from their points of origin to their assigned locations as well as connect with other cells, to facilitate vital communication among the cells<sup>[10-12]</sup>. These are processes that must occur within a tightly controlled time frame, such that each developmental stage has to be reached on schedule and in the correct sequence, since there is little potential for later repair, and the consequences may therefore be permanent<sup>[10-12]</sup>. Cowpea is consumed by different categories of human subjects in many communities in Africa; it is sometimes used to wean babies and this is one of the reasons why unspent phosphide residue should be prevented from mixing with grains.

That phosphide residue contaminated cowpea is capable of causing such toxic effect to the brain cells may be

buttressed by the effect of phosphine gas (derived from phosphides), Nath et al.<sup>[3]</sup> have identified that there might be neural/behavioural aspects of phosphine toxicity. In addition, phosphine gas has been linked with enhanced acetylcholine neurotransmission, this it does by suppressing acetylcholine esterase<sup>[13,14]</sup>. The toxic effects of many other pesticides have been established to affect the brain, for example the primary target of organophosphate (OP) insecticides is the enzyme acetylcholinesterase (AChE), which hydrolyses the neurotransmitter acetylcholine in not only the peripheral but also in the central nervous system. Organophosphates containing a P = O moiety are effective inhibitors of AChE, inhibition of AChE leads to accumulation of acetylcholine at cholinergic synapses, thereby causing over-stimulation of muscarinic and nicotinic receptors. This is a molecule (acetylcholine) that plays important role in brain development; acetylcholine and other neurotransmitters play unique trophic roles in the development of the CNS<sup>[15]</sup>; it may then be inferred that inhibition of AChE and the resulting accumulation of acetylcholine may disturb this developmental processes. Many of the features of "cholinergic syndrome" associated with this pesticides which are headache, drowsiness, dizziness, confusion, blurred vision, slurred speech, ataxia, coma, convulsions and block of respiratory centre<sup>[16]</sup> can also be linked with these morphological alterations observed in the brain histology of phosphide residue treated rats, an indication that a small amount of phosphide remains in the residue. This may be the reason why manufacturers of phosphide always discourage grain contamination by phosphide residue. That the abnormality in neurotransmitter acetylcholine may be involved in abnormal/morphologic changes in the brain cells may be evident from our findings in which congestion of the meninges was observed in treated rats, many abnormal brain presentations have been linked with lack of hydrolysis of acetylcholine.

Although many of these morphologic changes may be linked to inhibition of cytochrome c oxidase, of the mitochondrial electron transport chain, which is believed to be the primary site of action for phosphine<sup>[17-19]</sup>. The involvement of free radical generation cannot be discounted since 50% inhibition of cytochrome c oxidase is sufficient for generation of superoxide anions<sup>[19]</sup> have also buttressed this, indicating that increased lipid peroxidation in rat brain is always associated with aluminum phosphide ingestion. The study of Hsu et al.<sup>[20]</sup> was focused on phosphide-induced oxidative damage in rats and antioxidants as candidate protective agents revealed that when male Wistar rats were treated ip with phosphide at 2 mg/kg and thirty min later the brain, liver,

and lung were analyzed for glutathione (GSH) levels and lipid peroxidation (as malondialdehyde and 4-hydroxyalkenals) and brain and lung for 8-hydroxydeoxyguanosine (8-OH-dGuo) in DNA. Phosphide caused a significant decrease in GSH concentration and elevation in lipid peroxidation in not only the brain (36-42%), but also in lung (32-38%) and liver (19-25%). The abnormal lung presentation (severe congestion at the interalveolar septum, with diffuse hemosiderosis) in rats in the contaminated group may be as a result of combined toxic effect of phosphide (oxidative stress and cytochrome c oxidase)

Many of the common neurological changes like ataxia, stupor, tremors and convulsions have been observed following aluminum phosphide poisoning. Acute hypoxic encephalopathy has also been linked with exposure to aluminum phosphide, which may proceed to death as a result of complete depression of the central nervous system and paralysis of the respiratory centers of the brain<sup>[21]</sup>. Muscarinic effect which commonly present as diarrhea<sup>[22]</sup>, which has also been reported for carbamate insecticides, though absent in these rats is one of the most common complaints in human subjects in Nigeria post-cowpea consumption.

To further support the possible occurrence of brain abnormality in consumers of phosphide powder residue contaminated cowpea is the fact that there are reports to confirm that many of the workers who handle the fumigation process, who are exposed to various degrees of phosphide (phosphine), had manifested one or more neuropsychiatric symptoms such as anxiety, impotence and easy fatigue. With about 50% of the subjects (workers) recruited for the study showing hyperreflexia, polyneuropathy, lumbar radiculopathy, and cervical myelopathy, as well as anxious mood, impaired attention, and psychomotor stimulation. In addition, EEG recordings showed abnormal findings in 17.4% of the subjects, and this was mainly observed with longer exposure<sup>[23]</sup>, this therefore suggests that many of the histologic presentations observed in the rats exposed to phosphide powder residue may also present in human subjects who consume phosphide powder residue contaminated cowpea.

In conclusion, the effects of phosphide residue on important organs of the body are not diminished from those that have been earlier observed through the use of aluminum phosphide itself. This study shows that the erroneous belief among illiterate grain merchants that unspent phosphide residue may be harmless should be discarded and unintentional phosphide residue contamination of cowpea should be guarded against.

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