INVESTIGATION OF ACUTE AND SUB-CHROFIC TOXICITY
OF TWO ORGANOTIN COMPOUNDS IF THE RAT

BY

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DECLARATION

I hereby declare that the work presented in this thesis was carried out by me. No previous submission of it has been made elsewhere for a higher degree. The works of other researchers are fully acknowledged and referred to accordingly.

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DEDICATION

The thesis is dedicated to my little daughter, Aisha and her aunt, Hafsat.

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ABBREVIATIONS & SYMBOLS

ATP	Adenosine triphosphate
EU	Eusinophil
g or G	Gramme
Het	Haematocrit
lymp	lymphocyte
Mono	Monocyte
Neu	Neutrophil
nm	Nanometer
peros	Peroral
PPm	Parts per million
RBC	Red Blood Cells
Sn	Tin
TBTA	Tributyltin Acetate
TBTO	Tributyltin Oxide
TPTA	Triphenyltin Acetate
WBC	White Blood Cells
$>$	Greater than
$\leq$	less than or equal to
$\geq$	greater than or equal to

CHAPTER ONE

1.0 LITERATURE REVIEW AND STUDY OBJECTIVES

1.1 Background Information

Organotin compounds are a group of synthetic chemicals formed from the union of tetravalent tin atom and carbon compounds. These group of compounds were first introduced in the mid-1960s, ten years after their fungicidal property was recognized. Four classes of organotin compounds are generally in use; these include the mono-, di-, tri- and tetra-organotins. The general chemical formulae for these compounds are: $RSnX_3$ R_2SnX_2, R_3SnX and R_4Sn, where R is usually the butyl, octyl, cyclohexyl or phenyl group, and X a chloride, fluoride, oxide, acetate, hydroxide or carboxylate. Among these compounds, the di-, and tri-organotins have the most widespread field application. The tri- and tetra-organotin compounds are more toxic than the others.

Two of the most important tri-organotins in use for food preservation are the tributyltin-acetate (TBTA) and triphenyltin acetate (TPTA). At the Ahmadu Bello University Zaria, these two compounds were synthesized, tested, and found to be effective against the fungus which causes yam-rot (Bonire, 1977; Estevo, 1984 and Lodhi, 1985). This finding is of great importance in agriculture since yam is a staple Nigerian diet. Literature reports indicate that these types of compounds may be moderately toxic to mammals (Poller, 1970); Barnes *et al.*, 1958; Stoner, 1966 and Klimmer, 1963). These compounds have also been shown to produce histopathologic lessions in numerous mammalian organs (Klimmer, 1964; Pelican and Cern, 1968; FAO/WHO, 1971; Mijatovic, 1972 Guardascione and di Bosco, 1967; Horacele and Domicile, 1970).

1.2 Synthesis of Organotin Compounds

Organotin compounds are formed when tin, at oxidation four state, react with carbon in the appropriate amount. The four commercial methods in use for the manufacture of these compounds are Grignard, Wurtz, Aluminium alkyl and the Direct method (Vander kerk, 1970). The first three methods start by preparing tetraorganotin compounds (R_4Sn) from stannic chloride as shown in the schematic below:

(Eq.1.1) $SnCl_4 + 4RMgCl \longrightarrow R_4Sn + 4MgCl_2$.

(Eq.1.2) $SnCl_4 + 4RCl + 8Na \longrightarrow R_4Sn + 8NaCl$.

(Eq.1.3) $3SnCl_4 + 4R_3Al \longrightarrow 3R_4Sn + 4AlCl_3$.

After this initial steps, the second steps involve the preparation of organotin compounds of lower organic substituents from the three processes. This step can be carried out by various methods. One method is the comproportion or redistribution process, which is established. as the most efficient. In this method stoichiometric amount of tetraorganotin and stannic chloride are heated together at temperatures above 120°C to produce mono-, di-, and/or tri-organotin halides (Vander Berghe and Vander Kalen, 1966), see equations below.

(Eq. 1.4) $3R_4Sn + SnCl_4 \longrightarrow 4R_3SnCl$.

(Eq. 1.5) $R_4Sn + SnCl_4 \longrightarrow 2R_2SnCl_2$.

(Eq. 1.6) $R_4Sn = 3SnCl_4 \longrightarrow 4RSnCl_3$

The fourth method of production, the direct synthesis method, involves the heating together at temperatures above 150°C, tin metal and alkyl halides. A suitable catalyst is required to accelerate the rate of product formation.

The organotin halides are the starting materials in the preparation of other organotin compounds (Pollar, 1970; Anderson *et al.*, 1954). The organotin acetates (tributyltin acetate and triphenyltin inclusive) are prepared by mixing an organotin halide and silver acetate in diethyl ether. The reaction principle is equated below:

(Eq. 1.7) $n\text{-}Bu_3SnCl + CH_3COOAg \dashrightarrow n\text{-}Bu_3SnO,COCH_3, + AgCl,$

1.3 Physical and chemical Properties of organotin Compounds

Organotin compounds have poor solubility in water but are readily soluble in many of the common organic solvents. The solubility in water at ambient temperatures is in the order of 5-50mg/litre (WHO, 1980). With regards to chemical reactivity, these compounds are susceptible to attack by nucleophilic and electrophitic agents. Thus they may undergo hydrolysis, acidic and basic attack, and halogenation.

Stability of the compounds.

The organotin compounds can be degraded chemically, biochemically or by physical agents. The rate of degradation however, vary with environmental conditions. For example, hydrolysis occur only at extremes of pH (≤ 1 or ≥ 13), in the absence of chemical or physical catalysis (WHO, 1980). Solvolysis on the other hand occur mainly in cases of extreme laboratory conditions. The half-life of such reactions may range from one minute to upwards of about 100 days (Bassinadale, *et al.*, 1971; Roberts and El-Kaissi, 1968). In cases of photochemical decomposition, the reaction yield is higher at the lower wavelengths. The photolytic decomposition of triphenyltin acetate has been observed under laboratory conditions (Chapman and Price, 1972).

The rate of organotin decomposition vary considerably from one compound to another. For the triphenyltin compounds the half-time is between a few days to 140 days under environmental conditions (Freitag and Roche, 1974b). The half-time is shorter for the diorganotins (Mazaev *et al.*, 1976). Bruggemann and Klimmer (1964) and Bruggemann *et al.*, (1964a and b) achieved the complete degradation of triphenyltin acetate within the fifth week of applying it to sugar beet leaves at a concentration of 2470mg/kg. Other authors (Cenci and Cremonini, 1969) reported the degradation of triphenyltin acetate and hydroxide in 3-10 days and 3 days respectively, when the compounds were mixed with soil at a concentration of 80mg/kg of soil. In all the above cases the participation of microorganisms in the process were not established.

Products of Degradation

In a study by Akagi and Sakagami (1971) triphenyltin chloride solution yielded triphenyl-, diphenyl-, and phenyltin in addition to inorganic tin compounds, within 6 hours of exposure to ultraviolet light. Similarly, irradiation of trialkyltin cmpounds yielded a mixture of di- and mono alkyltin compounds, and inorganic tins within the same time. Triphenyltin acetate irradiated at 60 hours at a wavelength of 235nm yielded 10 percent of unchanged triphenyltin compound, 30 percent diphenyltin, 18 percent phenyl compound, and about 45 percent inorganic tin in the form of hydroxides and hydrated oxides. (Chapman and Price, 1972; Barnes *et al.*, 1973). Mazaev *et al.*

(1976) and Sheldon (1975) studied the degradation of some tri- and di-butyltin compounds in water. They found the half-time for degradation to be 1.14 days to 18.2 days at 20°C. They also found tin (IV) oxide as the final degradation product. In this experiment the diorganotins were found to have shorter degradation time when compared to the triorganotins.

1.4 Spectrum of Activity and Uses of Organotin compounds

1.4.1 Antifungal Action

Many Organotin compounds have been found to be fungicidal (Vander Kerk, 1954; Noto *et al.*, 1961; Poller, 1970) Tri-n-butyltin oxide is effective agaisnt the rice blast causing fungus *Pericularis oryzae* (Tamura and Hirokuni, 1965). Triphenyltin acetate has an effect on the fungus species causing potatoes and sugar beet diseases (Hartel, 1958). Recently both compounds have been found to be effective against yam rot causing fungi: *Fusarium* spp, *Candida albicans* and *Penicillium oxalicum* (Bonire, 1977; Esievo 1984; Lodhi, 1985).

The fungicidal activity of organotin compounds depend on the organic groups attached to the tin atom. The maximun activity is found with Triorganotins (Poller, 1970). The magnitude of fungicidal activity of Tri-n-butyltin and Triphenyltin acetates are comparable to that of organomercury compounds which are known to be the most potent fungicides up to date. More recent work by Adaudi (1983) has further confirmed this observation.

1.4.2 Bacteriostatic Action

Some organotin compounds have pronounced bacteriostatic properties. Trialkyl and Triaryltin compounds are very effective against gram positive *Staphylococcus aureus* in concentrations of 1-16 parts per million (ppm) (Pollar , 1970) or 25 ppm (Esievo, 1984). However, they have low activity against gram negative bacteria.

Bacteriostatic activity has also ben found among the dialkyl and tetraalkyl-tin compounds. The dialkyl-tins cause growth inhibition of several gram positive and gram negative bacteria. Diethyltin dichloride in particular, inhibit the growth of *E. coli*. The mode of bacteriotstic action is similar to that of arsenite.

1.4.3 Insecticidal Action

The insecticidal activity is largely confined to the triorganotin compounds. Some organotin compounds, in decreasing order of insecticidal properties, are: trimethyltin acetate, bistributyltin oxide, triphenyltin acetate, tributyltin acetate, diethyltin oxide and dibutyltin oxide (Pollar, 1970)

The mode of insecticidal action is said to depend on the type of organotin. Triphenyltins are said to act as stomach poisons rather than contact insecticides, where as tributyltin oxide act as fumigant (Pollar, 1970). Triphenyltin acetate and hydroxide were reported to exhibit a selective antifeedant effect towards certain insects. This property makes them potentialy useful for crop protection (Anne, 1984).

1.4.4 Miscellaneous Effects

The organotin compounds exhibit other effects. Some tributyltin compounds are reported to be highly effective as rodent repellants (Anon, 1967). and are used for impregnating cable sheaths to protect electrical and telephone cables against gnawing by rodents. Tributyltin oxide (TBTO) and some triphenyltin compounds are effective in controlling the snails which act as intermediate hosts for the parasites causing the tropical disease known as bilharziasis (Vander Kerk, 1970).

Dibutyltin dilaurate (Butyrovate) has been found to be effective against some intestinal worms of chickens, and has been used as antidote for that purpose (Vander Kerk, 1970). Triphenyltin has been reported to have a potent immunosuppressive effect (Verschumre *et al.*, 1970).

1.4.5 Uses of Organotins

Organotin compounds were developed primarily for use in the industry, and about 70 percent of the total production of the compounds are used to heat-stabilize poly-vinyl chloride (PVC) in plastic industry (WHO, 1980). Of the four categories of organotin compounds, dialkyltin and monoalkyltin compounds are the most commonly used for this purpose (Vander Kerk, 1970). The dialkyltin are used to stabilize PVC plastics during manufacture and to prevent their degradation during use. They are also used as catalysts in the production of polyurethane foam products. The mono-alkyltins have a limited use as heat stabilizers for PVC plastic films.

The triorganotins are mainly used in agriculture as fungicides (Anne Marie Coles, 1984). Some are also used in the industry as biocides and surface disinfectants (Vander Kerk, 1970). Triphenyltin (fentin) acetate and hydroxide were the first to be used in agricuture. Triphenyltin acetate is used as fungicide on a variety of crops such as beans, celery, carrots, cocao, coffee, onions, potatoes, rice, sugar beet and many other valuable crops. It is also used as algicide and molluscide in padday rice field, and in fish ponds. Both triphenyltin acetate and tri-n-butyltin acetate are currently being proposed for use against yam rot (Bonire, 1977). Triphenyltin hydroxide is used against tuber blight disease in sugar beet, as well as rodent repellant. Tricyclohexyltin hydroxide has a strong miticidal property, and is used to halt the spread of phytophagous mites which damage fruits, cotton, vegetable and tea crops.

Industrial uses

Tributyltin compounds are particularly useful as a general industrial biocide and as surface disinfectants. Tributyltin oxide (TBTO) is used in antifouling paints for the purposes of wood preservation, and also for keeping clean the recirculating water of cooling towers (Vander Kerk

1970). It is incorporated in synthetic rubber for surface protection of the mamufactured products (Goodrich Company, B.F.U.S.3,426,473,1969). Some tributyltin formulations act as rodent repellants to protect cable sheats and electrical telephone cables (Anon, 1967), while other formulations are used as constituents of hospital and veterinary clinics disinfectants (Wanke, 1967). The tetraorganotin compounds have very limited industrial uses. Some tetralkyltin derivatives are used as intermediates in the manufacture of other compounds. Tetraphenyltin have been used as heat stabilizers for polychlorinated biphenyls which are used as transformer oil (Vander Kerk, 1970).

1.5 Metabolism Of Organotin Compounds

1.5.1 Absorption

Studies in animals have shown that the absorption of orally ingested organotin compounds varies considerably between different animal species. In general, the compounds with short akyl chains are more readily absorbed from the intestinal tract. Ethyltin trichloride was found to be poorly absorbed when a single dose of 25mg/kg was orally administered to rats. About 92 percent of this dose was eliminated in faeces within two days. Triphenyltin which was administered orally was also poorly absorbed by sheep (Herok and Ezotte, 1964) and cows (Bruggemann *et al.*, 1964a), but was well absorbed by the guinea pig and mice (Stoner, 1966).

Trialkyltin compounds are usually well absorbed through the dermal route. When a 20 percent fat solution of various triethyltin derivatives were applied to the skin of rats and mice for 10 minutes, they caused 100 percent death within 20-30 minutes (Ignatjeva *et al.*, 1968). In contrast, triphenyltin acetate did not penetrate the unbroken skin readily (Stoner, 1966).

1.5.2 Distribution

After absorption, organotin compounds are distributed into various animal tissues. Studies in rats and some other laboratory animals showed that the highest concentration of the chemicals are mostly found in the liver. In rats and mice given dibutyl-, and diethyl-tin intravenously, the highest concentration of the compounds were found in the liver and kidney tissues with the unchanged compounds being excreted in the bile (Barnes and Magee, 1958). When rats were fed with 11mg of triethyltin hydroxide over a period of 89 days 40 percent of the dose was distributed in the blood, 29 percent in skeletal muscle, 26 percent in the liver. Some quantities of the chemical were also found in the kidney, brain, heart, and spleen (Cremer, 1957). The same pattern of distribution was observed when the same compound was given intraperitoneally to the rabbit. However, some species differances have been observed in *in vitro* tests (Cremer, 1957).

Following an oral or intravenous administration of the radionuclide ^{113}Sn triphenyltin, to rats and guinea pigs the concentration of the radioactive tin was found in the brain, but the form in which the tin was present in the brain was not satisfactorily identified (Heath, 1967). When the ^{113}Sn-labelled triphenyltin acetate was administered orally to sheep at a dose of 10mg/kg per day the highest concentration of the ^{113}Sn was found in the liver, but the brain was also found to contain some measurable amounts of the toxicant (Herok and Gotte, 1964).

Trace amounts of tricyclohexyltin hydroxide were found in the tissues of the rats and dogs fed up to 12mg/kg body weight per day, for a period of 45 days to 2 years (FAO/WHO, 1971).

1.5.3 Biotransformation

Many organotin compounds are transformed in the tissues. In laboratory animals, tetra-, tri-, and di-substituted organotins are mainly transformed in the liver. Some metabolism in the gut was also observed with the diethyltin treated rats (Bridges *et al.*, 1967).

The metabolism of organotin principally involve the carbon-tin bond cleavage (destannylation), which involves dealkylation or deacylation of the compound. Triethyltin was identified as the metabolite in rats and rabbits given an intravenous dose of tetraethyltin (Cremer 1957 and 1958). Bridges *et al.* (1967) detected some dealkyltion products with diethyltin in rats which he attributed to gut and tissue metabolism. Dicyclohexyltin oxide and trace amounts of cyclohexyl stannoic acid were detected in the tissues of rats and dogs treated with tricyclohexyltin hydroxide (FAO/WHO 1971). Small amounts of inorganic tin compounds were detected in the milk of sheep treated with ^{119}Sn-labelled triphenyltin acetate. Stonner (1966) had observed that the compound was not metabolized in the body.

Recent studies have shown that a biological oxidation reaction is the major metabolic route of degradation for some organotins. Fish *et al.* (1975) obtained alpha-and beta-hydroxylated metabolites with tributyltin acetate using rat microsomal enzymes in an *in vitro* study. Kimmer *et al.* (1977)obtained the same result *in vivo* using a ^{14}C-labelled tetrabutyltin acetate in mice. The same author also abtained diphenyl-, and phenyl-derivatives with triphenyltin acetate in rat studies. He also observed that a carbon hydroxylation of cyclohexyl group is the major metabolic reaction for tricyclohexyltin compounds *in vitro*.

1.5.4 Excretion

The route of excretion of organotin compounds in laboratory animals depends largely on the type of compound. For example, ethyltin-trichloride was found to be excreted mainly in the urine when adminitered intraperitonially to rats, but diethyltin is eliminated in the faeces, urine and the bile (WHO, 1980; Bridges *et al.*, 1967). Triphenyltin acetate was excreted in urine and milk of lactating sheep when the animal was given the chemical at the rate of 10mg/day for 20 days (Herok and Gotte, 1964).

The rate of excretion of various organotins varies, and many compounds are slow to disappear from the organs especially the brain. In guinea-pigs, the half life of triphenyltin concentration in the brain was reported to be over 10 days, but in rats it was found to be 3 days (Heath, 1963 and 1967). For tricyclohexyltin hydroxide the biological half-life was found to be between 5 to 40 days in rats when the chemical was added in the diet for a prolonged period. The brain was observed to be one of the tissues from which the compound was removed slowly (FAO/WHO, 1971).

1.6 Acute toxicity of organotin compounds

1.6.1 Acute toxicity Values (LD_{50})

Many organotin compounds are poisonous. The acute toxicity values (LD_{50}) of trimethyl and triehyltin compounds are comparable to that of the well known poisonous chemicals (Neumann, 1967)

The acute and chronic toxicity of the organotins varies according to the number of organic groups attached to the tin atom. The lowest LD_{50} value, and hence the highest toxicity, is shown by compounds having 3-tin carbon bonds (Triorganotins). For example the (LD_{50}) of trimethyltin acetate and triethyltin-acetate, chloride and sulphate in rat is less than 10mg/kg (WHO, 1980; Neumann, 1967).

Toxicity is also dependent upon the nature of the organic group attached to tin. The most poisonous of these compounds are trimethyl- and triethyl-tin derivatives, even though no organotin compound is regarded as extremely toxic, that is having lethal value at 1mg or less (Neumann, 1967). All butyltins, high alkyltins, and phenyltins belong to the category of moderately toxic compounds LD_{50} value of 50-500mg/kg, and practically non toxic organotins, with the LD_{50} value of 5-15gm/kg (Spector, 1955). The n-octyl organotins are the least toxic (Klimmer and Nebel, 1960). There is a wide variation in the LD_{50} figures reported for triphenyltin and tributyltin acetate in rats (Poller, 1970; Barnes *et al.*, 1954; Esievo, 1984; Lodhi, 1985).

1.6.2 Acute toxicity symptoms

Typical signs of acute toxicity have been reported in many animal species following acute doses of various organotins. With triphenyltin acetate the typical symptoms of acute toxicity in rats are flaring of the nostrils, rapid and shallow breathing, and apnoea (Esievo, 1984). Tri-n-butyltin acetate produced similar symptoms in rats, in addition to instability in gait, due to hind limb extension, and chronic convulsions (Lodhi, 1985). In mice given 500mg/kg tributyltin as acetate, chloride, benzoate or oleate orally the typical signs of acute toxicity were seen four hours following the administration. But in the case of laurate, the signs were delayed for a further four hours. The acute toxicity symptoms following a 5mg/kg triehyltin sulphate in rats include prostration, flaccid paralysis and encephalopathy (Stonner *et al.*, 1955). For tetraethyltin series the major signs observed include muscular weakness and paralysis, followed by respiratory failure. The development of these signs are said to be slow (Canjolle *et al.*, 1954).

Acute doses of organotins also produce tissue lessions in animals. A single dose of 400mg/kg butylstannoic acid, butylthiostannoic acid, and butyltin trichloride in mice produced fatty degeneration of the renal cortical tubular epithelia, gastrointestinal haemorrhage, lymphatic tissues necrosis, and liver enlargement in mice (Pelikan and Carny, 1970b). Single oral doses of dibutyltin dichloride at 50mg/kg body weight produced congestion and inflammation in the lower part of the bile duct in rats while higher doses produced necrotic changes in the liver of mice and rats (Barnes and Magee, 1958; Gaunt, 1968). Bile duct injury has been observed also in rats and rabbits following dermal application of dibutyltin dichloride at 10mg/kg body weight (Barnes and Magee, 1958). Pulmonary congestion and oedema were reported in rats after a single intravenous dose of 20mg/kg diethyl-, dipropyl-, diisopropyl-, and dipentyl-tin dichloride, or 10mg/kg dibutyltin dichloride (Barnes and Stonner, 1958). Some dioctyl compounds at an oral dose of 4000mg/kg body weight produces changes in germinal centres of the spleen and steatosis of hepatocytes in mice (Pelikan and Cerny, 1970a).

1.6.3 Post-mortem Lesions

Acute doses of some trialkyltin compounds are reported to cause a characteristic lesion in the central nervous system and some other tissue changes. Hedges and Zaren (1969) described papilloedema in monkeys following a single intraperitoneal dose of triethyltin acetate at 0.1mg/kg body weight. A single oral dose of tributyltin acetate, benzoate, chloride, laurate or oleate at 50mg/kg body weight produced fatty infiltration of the liver in mice (Pelikan and Cerney, 1968a). An aqueous solution of bis (tributyltin) oxide produced slight local irritation when applied to the shaved skin of rats at lower doses (0.36-0.9mg/kg), but at higher doses skin necrosis was observed (Pelikan and Cerny, 1968 and 1967). Skin reaction was also reported in rats given a single dose of 15mg/kg tripenyltin acetate (Klimer, 1964). Both bis (tributyltin) oxide and triphenyltin hydroxide were reported to cause severe eye irritation and sometimes corneal opacity after brief exposure (Pelikan and Cerney, 1968; Mark *et al.*, 1969).

1.7 Sub-Acute and Chronic Toxicity of Organotin Compounds

1.7.1 Sub-acute and Chronic Toxicity Values

Subacute and chronic toxicity values of organotin compounds are relatively less than the acute toxicity. The lowest oral daily dose of dibutyltin sulphate causing intoxication in rats, during a 7 months chronic exposure was found to be 0.1mg/kg (Mazaev and Korolev, 1969). For triphenyltin the lowest dietary level causing death in rats within 17 days of daily exposure was reported to be 50mg/kg, and the lowest level causing growth retardation in the same animal was found to be 25mg/kg as compared to 5mg/kg of triethyltin hydroxide (Klimmer, 1964). The lowest dietary level of tricyclohexyltin hydroxide that resulted in loss of apetite, weight, and subsequent death in dogs is 12mg/kg. This is compared to the intravenous LD_{50} of 14mg/kg in the animal. The lowest level of the same compound causing toxic effects in rats was found to be 25mg/kg by the same authors (FAO/WHO, 1971).

1.7.2 Sub-acute and chronic toxicity

Subacute and chronic exposures to organotin compounds have been shown to produce many effects in experimental animals. When 50mg/kg dibuyltin dichloride was given orally to rats for 3 successive days, severe congestion and inflammation of the proximal part of the bile duct and portal vessels, as well as necrosis of the liver were observed (Barnes and Magee, 1958; Gaunt *et al.*, 1968). In another experiment, Mazaev and Koralev (1969) reported changes in the kidneys in rats treated with dibutyltin dichloride at 0.1 and 1.0mg/kg body weight. Gaunt *et al.* (1968) also reported mild anaemia, growth retardation and decreased food intake in rats kept on a dietary level of 80mg/kg dibutyltin dichloride for 90 days.

Oral administration of dibutyltin disulphide at 0.1mg/kg body weight for a period of 6 months, caused a decrease in the number of erythocytes and reticulocytes and an increased percentage of chromosomal aberrations in bone marrow cells, as well as increased mitotic index in small intestinal mucosa of rats (Mazaev and Sleping, 1973). Dose dependent reduction in the weight of the thymus and the peripheral lymphoid organs was seen in rats fed with dioctyltin dichloride and dibutyltin dichloride at a dietary level of 80 and 150mg/kg for 6 weeks (Sienen, *et al.*, 1977). Similar effects on rats lymphoid organs were seen with diethyltin dichloride and diproyltin dichloride, but no such effects were observed with the other dialkyltins or with tri- or tetra-alkyltin compounds (Sienen *et al.*, 1977).

Triphenyltin acetate at a dietary level of 10mg/kg body weight produced fatty degeneration of the liver in the guinea pigs, when administered over a 2-year period. The same drug also caused a dose-related inhibition of growth rate in the same animal species when given at dietary levels exceeding 5mg/kg for 2 years (FAO/WHO, 1971). Decrease in the percentage of haemoglobin and in the number of erythrocytes were reported in guinea pigs treated with triphenyltin acetate at a dietary level of 10mg/kg for 4 months. A decrease in haemoglobin and leukocytes were also seen in rats after they were kept on a 50mg/kg levels of triphenyltin hydroxide for 12 weeks (FAO/WHO, 1971). In one experiment with guinea pigs, a dietary level of 15mg/kg triphenyltin acetate for 77 days produced a decrease in plasma cells in the spleen and in the mesenteric, cervical, axillary and popliteal lymph nodes. After feeding the same dose to female guinea pigs for 104 days, a reduction in immune response to tetanus toxoid stimulation was observed (FAO/WHO, 1971). In another experiment a reduction in the number of lymphocytes and leukocytes, as well as histological changes in the lymphatic tissues were observed in female guinea pigs that were on dietary levels of 5mg/kg, triphenyltin acetate, and in males on 10mg/kg, for 12 weeks (WHO/FAO, 1971

A dietary level of triphenyltin hydroxide of 5mg/kg for 90 days, caused a dimunition in the size of the testes and a less advanced maturation of the germinal epithelium in rats. However, a three-generation study of the dietary intake failed to demonstrate any adverse effect on the reproductive indices(FAO/WHO, 1971). In another experiment at a dietary level of up to 200mg/kg over a 276 day period, Gaines and Kimbrough (1968) observed a reversible reduction in fertility in male rats which was considered to be due to a pronounced decrease in food intake. No effect on reproduction was obtained in experiments with other trialkyltin compounds FAO/WHO, 1971).

Triethyltin compounds have been observed to cause depression of the central nervous system. A dietary level of triethyltin hydroxide of 20mg/kg caused a reversible hind legs weakness in rats. A level of 80mg/kg in the same species caused tremors resembling those seen in acute trimethyltin poisoning (Stoner *et al.*, 1955). Triethyltin sulphate administered to new-born rats and their mothers at a dose of 5mg/litre in drinking water for four months resulted in the paralysis of the posterior limbs, severe cerebral oedema and status spongiosus of the white matter in the mothers (Suzuki, 1971).

Trichlorohexyltin hydroxide was reported to cause cholengitis, congestion of the glomeruli and mild toxic nephrosis in rats given the drug at a dietary level of 400mg/kg (Shirasu, 1970). The drug when given to rats at 25mg/kg daily for 19 days also produced intra- and extra hepatic cholengitis, toxic nephrosis and gastroenteritis (FAO/WHO, 1971). A slight reduction in weight was also reported in rats receiving a dietary level of 25mg/kg of the drug for 90 days (Shirasu, 1970).

1.8 Objectives Of The Current Project

Two organotin compounds tri-n-butyltin acetate (TBTA) and triphenyltin acetate (TPTA) have been synthesized locally at the Department of Chemistry, Ahmadu Bello University. These compounds were found to possess fungicidal action. Current studies in our microbiology laboratory have suggested that these compounds could be useful in controlling yam rot. Since yam is a popular Nigerian food crop, it would be necessary that the chemicals are evaluated for human safety before use. Some preliminary toxicity studies have been done on the two compounds (Esievo, 1984; Lodhi, 1985). This project however, is the first attempt at intensively investigating these two chemicals for their mammalian toxicity. Acute and sub-chronic toxicity studies would be done in the rat to determine the effect of a high level one-time exposure to these compounds as well as a moderate but prolonged exposure to the compounds as would be the case in diets. Histopathological and post-mortem analysis would be done to determine if there are subtle but significant damages that a prolonged use of these compounds in food crops may provoke. The study is specifically designed to:

i) determine the median lethal dose (LD_{50}) of TPTA and TBTA in rats,

ii) observe and record the clinical signs and post-mortem findings of treated rats, and

iii) use the toxicological data obtained from the study to assess the safety of these compounds in food crops.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.10 MATERIALS

2.11 TEST CHEMICALS

Tri-n-butyltin acetate (TBTA) and triphenyltin acetate (TPTA) are the two organotin compounds that were tested. The chemicals were synthesized at the Department of Chemistry, Ahmadu Bello University (ABU) Zaria, Nigeria.

2.12 OTHER CHEMICALS

Tween 80 (Polyoxyethlene sorbitone monoleate) was used to suspend the test chemicals. It was obtained from Sigma Chemical Company, St. Louis Missouri, U.S.A.

Formalin 10% (May and Baker Dagenham England) was used for fixing the tissues prior to their processing.

Absolute alcohol (Hopkin Williams Chadwell - Heath Essex, England) was used in processing the tissues.

2.13 TEST ANIMALS

The test animals were adult ablino rats (males and females) which were inbred in the Animal House, Faculty of Pharmaceutical Sciences, Ahmadu Bello University. Additional animals were obtained from the Faculty of Veterinary Medicine, A.B.U. All the rats used were first observed for two weeks prior to admission into the experiments. Any animals that showed apparent ill health were excluded from the experiment.

2.14 ANIMAL FEED

Commercial Pfizer feed pellets (Pfizers, Ikeja, Lagos Nigeria) were used to feed the rats throughout the period of study.

2.15 APPARATUS

1. Pestle and Mortar was used for the preparation of test chemical solutions used for the experiments.

2. A Vortex Mixer 220/250 V A.C. (stuat U.K.), was used to shake each individual solution prior to its injections. This is to allow for more even dispersion of the test chemical solutions.

3. 1 and 2ml syringes and needles were used for oral injection of the test chemical solutions. The needle (21½G) was bent at 90°, its sharp end was cut off and covered with a rubber (Polyethlene) tubing to allow for easy and safe injection into the oesophagus of the test animals. The other end was connected to the syringe containing the test chemical solution.

4. 20ml Universal bottles were used to store prepared solutions of test chemicals and tissue specimens.

5. EDTA bottles (seward, U.K.) were used in collecting blood for haematological test.

6. Avery balance (W. and T. Avery limited - Birmingham, England) was used in weighing the experimental animals.

7. Mettler balance (P. 162 Gallen Kamp U.K.) was used for weighing the TBTA and TBTA, as well as for weighing organs and tissues of posted animals.

8. Non reactive and water soluble red markers (Steadtler, Germany № 352) were used to mark each

individual rat on the tail for the purpose of identification

9. Plastic cages (53cm x 38cm x 20cm, E.K. - Brown Ltd, London, U.K.) were used to cage the rats. 5 animals of same sex were kept in each cage to give enough room, and to avoid pregnancy during the peiod of experimentation.

10. 250ml and 500ml plastic bottles were used to keep drinking water for the experimental animals. The water was changed after every 2 days.

2.20 EXPERIMENTAL METHODS

2.21 PREPARATION OF TEST CHEMICALS SOLUTIONS

Solutions of the test chemicals were prepared by triturating a weighed quantity of each compound with a measured volume of 5% Tween 80 V/V using pestle and mortar. The prepared solutions were stored in 20ml Universal bottles, and kept at room temperature for a maximum of 7 days after which new ones were prepared again.

2.22 <u>ORAL ADMINISTRATION OF TEST CHEMICALS</u>

The test chemicals were administratered orally by inserting the curved end of a needle, coverd with polythene tubing into the oesophagns of the rat, and injecting the test chemical solution contained in the syringe that was connected at the other end of the needle. For acute tests the administration of each dose was done once. In case of sub-chronic test the administration was done 3 times in a week for 5 consecutive weeks.

Five increasing dose levels of each compound were used for the exploratory (Preliminary) and final acute tests. 2 and 4 rats per dose level were used for the first and second exploratory tests respectively, and 10 (5 males and 5 females) were used for the final test, 10 control rats (males and females) each received 0.5ml 5 percent Tween "80" orally. The animals were observed for 24 hours for acute toxicity signs and symptoms.

Three geometrically selected dose levels of each compound were used for the sub-chronic test. A 5 percent Tween "80" was used as control. Ten animals (5 males and 5 females) were used for each dose level and also for the control, as in the acute test. The volume of Tween "80" injected is the maximum volume given as test chemicals solution. The animals treated were observed daily for 5 weeks for sub-chronic toxicity symptoms and death. Every week, body weight and daily feed consumption of each individual rat was recorded. Post mortem was performed on all animals used for the sub-chronic test and on some dead animals used for acute test.

2.23 DETERMINATION OF MEDIAN LETHAL DOSE (LD_{50})

The *per os* median lethal dose of each test chemical in rats was determined using standard procedures (Aliu and Nwude, 1982). Two pilot tests were first carried out by giving 5 increasing doses of each of the test chemicals to small groups of rats of the same sex. The doses were selected logarithmically, and the least dose that killed all animals in a group in the pilot tests one and two were used as focal points for the determination of dose range to be used in subsequent experiments.

In the first pilot test, a pair of rats each received one of the five dose levels selected at 0.6 log (factor 4) interval, and in the second test, 2 pairs each received a dose selected using a factor of 1.4 (log 0.15).

The final tests were carried out using a factor of 1.3 (log 0.11) and 1.2 (log 0.08) for TBTA and TPTA respectively. Fifty normal healthy rats (average weight, 160g) were used for the test of each chemical. The animals were divided into 3 groups of 10 rats (5 males, and 5 females). Each group was given a predetermined dose of the appropriate chemical. A control group of 10 rats (5 males, 5 females) received 0.5ml (each) of 5 per cent Tween '80". The number of animals that died within 24 hours were noted for each group. The surviving rats were observerd for 14 days for delayed toxicity symptoms.

The LD_{50} was calculated by Arithmetic method of Karber (Aliu and Nwude, 1982), and by Miller and Tainer (1944) graphic method.

2.24 GROSS AND HISTOPATHOLOGICAL STUDIES

1. Necropsy

Post-mortem examination was performed on some animals that died during the acute tests and all the animals used for sub-chronic experiment. These studies were conducted with the assistance of a veterinary pathologist in the Department of Veterinary Pathology and Microbiology, Ahmadu

Bello University Zaria. Gross pathological changes were recorded following visual examination of the posted animals.

2. Tissue Processing and Sectioning

Fresh samples of the brain, liver, kidney, lungs, heart, intestine, stomach and spleen from both treated and control rats were fixed in 10 percent formal-saline. Tissue slides of 3-4cm thickness were cut from each organ for procesing. The cut tissues were transferred to the automatic tissue processor where they were further fixed in 10 percent buffered formal-saline for two hours and dehydrated for 2hr in each of an ascending grade of alcohol (70%, 90% and 100% v/v). The dehydrated tissues were then cleaned in xylene for 2hr and the tissues impregnated in molten paraffin. This lasted for two hours, after which the tissue slices were left to cool. The sections were then trimmed and sectioned on the microtome at 5 microns. The sections were then flooded in a warm water bath, attached to slides and dried on a hot plate and stained.

3. Staining of Sectioned Tissue

Haematoxylin and Eosin Stain (H&E stain)

The sections were dewaxed in xylene, and rehydrated in descending grades of alcohol: 100%, 90% and 70%. They were then stained in haematoxylin for 10min, differentiated in 1% acid alcohol, blued in Scott's tap water and stained in eosin for 3min. They were then rinsed and dehydrated in ascending grades of alcohol solution: 70%, 90% and 100% and finally, cleaned in xylene and mounted in Pox.

These slides were examined microscopically for lesions with the assistance of a Veterinary Pathologist at the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Photographs of any lesions of interest were taken for illustration and record keeping.

2.25 HAEMATOLOGICAL EXAMINATION

Haematological examinations were performed on all the rats at the end of the experiment, using standard methods. The blood for the tests was obtained by cervical decapitation of rats and draining sufficient quantity of blood into two separate bottles (EDTA and plain bottles).

Haematocrit (Hct) values were determined by the microhaematocrit method. Total Red Blood Cell (RBC) and White Blood Cells (WBC) counts were determined using the Coulter Counter. Differential count was performed according to standard procedure as described by Coles (1974). The sample blood smear was made by carefully a small drop of uncoagulated blood in the middle of a clean glass slide and spreading it with a spreader. This blood smear was air-dried and then fixed in ethanol for 5 miutes. It was then stained with Giemsa stain for 20 minutes, rinsed in tap water and allowed to air dry again. The differential count was done by examining the slide under a light microscope under an oil immersion lens.

The mean blood counts obtained for each treated group of rats was then compared with those of the controls.

2.26 <u>STATISTICAL ANALYSIS</u>

The statistical comparison of the mean organ/body weight as percentage ratios and the haematological values for each group of experimental rats and control group were computer analysed using the University of Minnesota Fortran computer, version 5-0-77/03.24. The comparative analysis was made using a Sudent t-test at a 5% ($P<0.05$) level of significance.

CHAPTER THREE

3.0 EXPERIMENTAL RESULTS

3.1 ACUTE TOXICITY TESTS

The results of the acute toxicity tests for TBTA and TPTA are as shown in Tables I to IV and Figures 1 and 2. The tables indicate the various doses of the chemicals administered per group of rats and the number of death recorded within 24hr of dosing. These data have been processed for the calculation of LD_{50} by two methods

Median Lethal Dose (LD_{50}) Calculation

The median lethal dose values per os of TBTA and TPTA were determined from these data by Karber (Aliu and Nwude, 1982), and also the Miller and Tainer (1944) methods. The values are 297.54 and 296.0, and 402.38 and 399.50mg/kg for TBTA and TPTA respectively. The Arithmetic Method value was calculated by substracting the sum of the product of the mean dead and the dose difference quotient from the least dose that killed all the animals. The graphic method involve plotting a graph of data, 10 to 90% effect, on logarithmic probability graph paper. The LD_{50} values were read off directly from the plots for TBTA and TPTA as the dosage values at 50% from the straight line fit plot.

Table I

Data from the dose of TBTA administered and the resulting Mortality Rate Recorded within 24hr Period.

Expt Rat Group	Dose Administered *per os* mg/kg	Differential	Animals Dead	Mean Dead	Dose Differential X Mean Dead
1	160		0		
		48		0,5	24
2	208		1		
		62,4		3	187,2
3	270,4		5		
		81,1		6	486,6
4	351,5		7		
		105,48		8,5	896,58
5	456,98		10		
					Total 1594,38

Calculation of LD_{50}

$$LD_{50} = 456.98 - 1594.38/10$$
$$= 456.98 - 159.438$$
$$= 297.542$$

The $LD_{50}(24)$ for TBTA (tri-n-butyltin acetate) was calculated to be 297.54mg/kg by the Arithmetic Method of Karber.

Table II

Data for the determination of LD_{50} of TBTA by the graphic method of Miller and Tainer

Expt Rat Group	Dose mg/kg *per os*	Animal Dead	Animal Survived	Percent Dead	Corrected Percent Dead
1	160	0	10	0	0,25
2	208	1	9	10	10
3	270,4	5	5	50	50
4	351,5	7	3	70	70
5	456,98	10	0	100	97,5

Corrected Percent Dead Factor:

$$\text{For } 0\% : \quad \frac{10(0.25)}{n}$$

$$\text{and for } 100\% \quad \frac{100(n-0.25)}{n} \;;$$

where n is equal to the number of animals in each group.

Table III

Data from the dose of TPTA Administered and the Resulting Mortality Rate Recorded within 24hr Period.

Expt Rat Group	Dose Administered *per os* mg/kg	Differential	Animals Dead	Mean Dead	Dose Differential X Mean Dead
1	250		0		
		50		0,5	25
2	300		1		
		60		2	120
3	360		3		
		72		4,5	324
4	432		6		
		86,4		8	691,2
5	518,4		10		
				Total	1160,2

Calculation of LD_{50}:

$$LD_{50} = 518.4 - 1160.2/10$$

$$= 518.4 - 116.02$$

$$= 402.38$$

The LD_{50}(24hrs) for TPTA (triphenyltin acetate) was calculated to be 402.4mg/kg by the Arithmetic Method of Karber.

Table IV

Data for the determination of LD_{50} of TPTA by the graphic method of Miller and Tainer.

Expt Rat Group	Dose mg/kg *per os*	Animal Dead	Animal Survived	Percent Dead	Corrected Percent Dead
1	250	0	10	0	0,25
2	300	1	9	1	10
3	360	3	7	3	30
4	432	6	4	6	60
5	518,4	10	0	10	97,5

FIG.1. Determination of LD50 of TBTA Using graph: Corrected % Mortality against Dose in mg/kg (Rat).

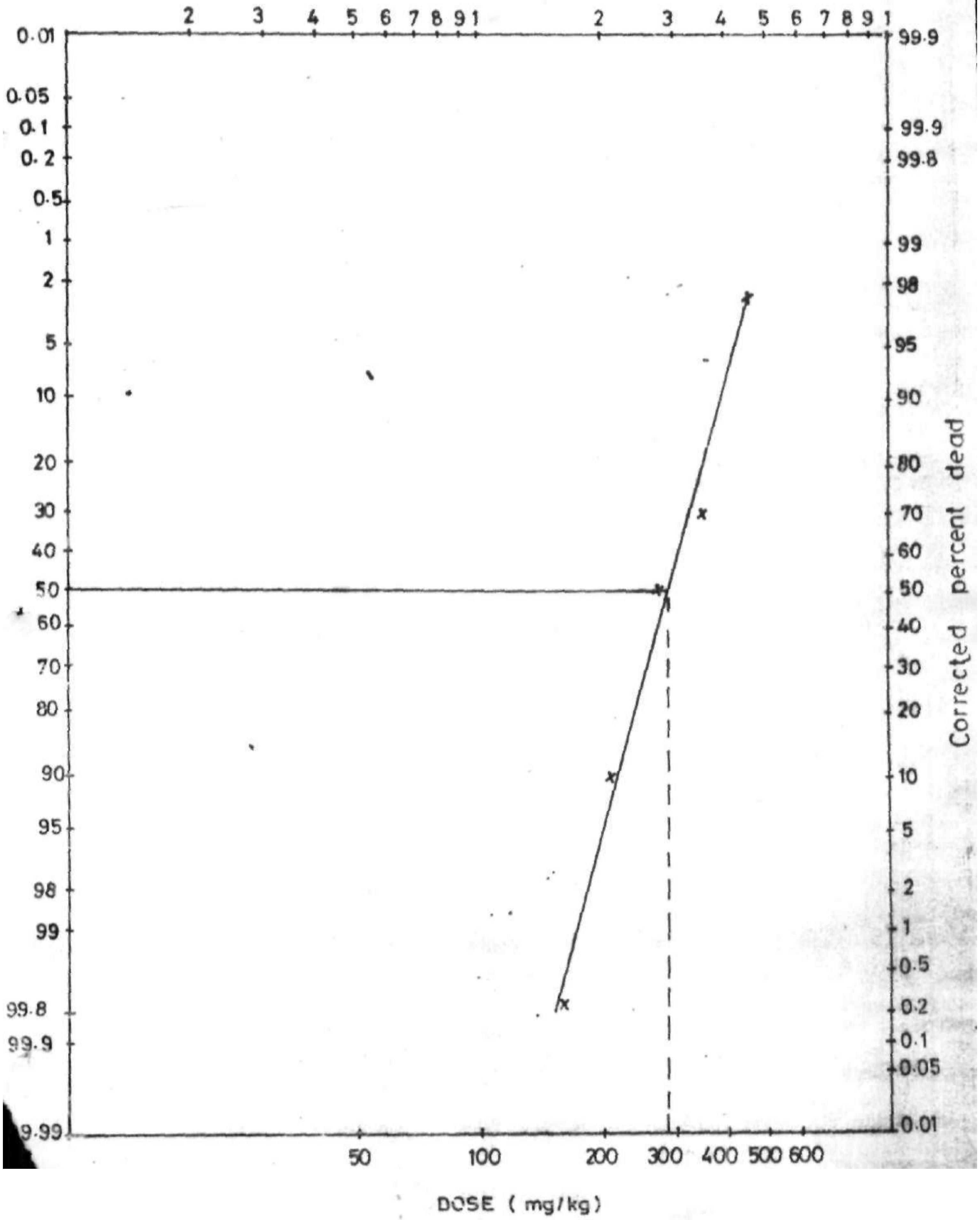
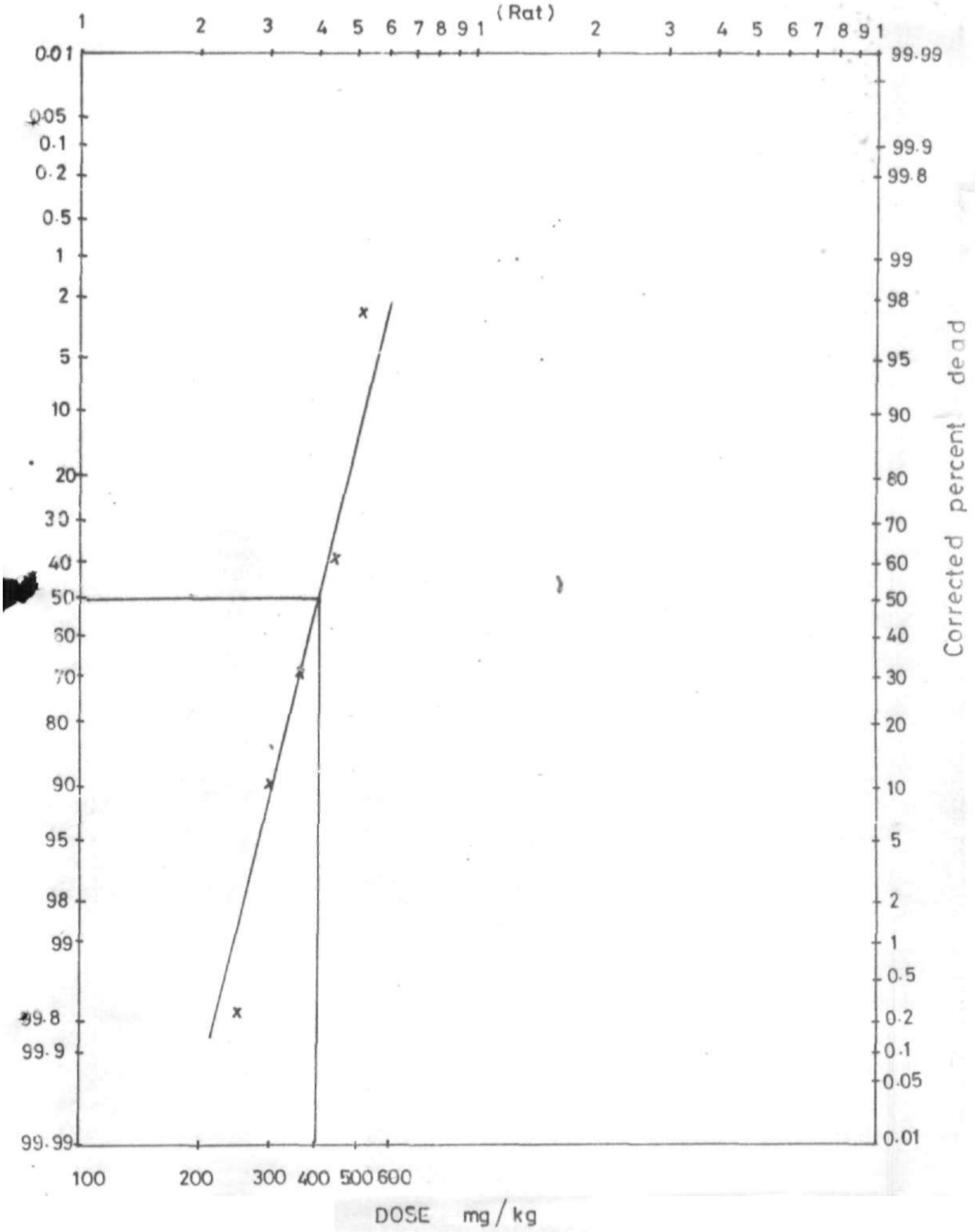


FIG 2. Determination of LD50 of TPTA Using graph: Corrected % Mortality against Dose in mg/kg (Rat)



3.1.2 CLINICAL SYMPTOMS AND SIGNS

Tri-n-butyltin Acetate

All rats orally dosed with tri-n-butyltin acetate - TBTA, 160mg/kg and above showed signs of dermal and gastrointestinal irritation. This manifested as excessive rubbing of the mouth and abdomen as well as hind limb stretching within one hour of oral injection of the chemical.

Other symptoms were also observed within 24hrs of TBTA administration. Sedation was seen in animals receiving doses above 160mg/kg; this was followed by rapid and shallow breathing, sweating, dyspnoea and finally death. Death started to occur 5-10hrs after drug administration in rats given 208mg/kg, and in those rats receiving higher doses. Death was immediately preceded by tonic hind limb extensions, and convulsions. There was also loss of appetite generally.

In the low dosed surviving rats, dyspnoea ceased within 2-3 days while sedation continued until death. In the high dose groups, incordination, loss of body reflex, severe occular inflammation, frequent and prolonged tendency to lying on the back were also observed in the surviving animals. Hair loss (alopecia, Fig. 3), scaly limbs and soft faeces were also seen in addition to the above signs in two surviving rats given 400mg/kg during one of the pilot tests. However, this sign was not observed in subsequent testing. The hair loss was most conspicuous on the 10th day after the administration of the compound.

Only the lower dose groups (160 to 208mg/kg) rats recovered completely after 24hrs.

Triphenyltin Acetate (TPTA)

Signs of acute toxicity similar to those seen within the first 24hrs of TBTA administartion, were also observed with Triphenyltin acetate. However, with TPTA the signs were less severe, and they had longer times of onset and duration of persistence. Time before death was also much more prolonged with TPTA, occuring after 10-16hrs of oral administration. Also unlike TBTA, marked excitation was observed with the lower non-lethal doses, and depression was observed with high lethal doses of TPTA.

In the surviving rats ceasation of dyspnoea followed by prolonged sedation was also observed as in TBTA. Most of the surviving animals recovered completely within 2 weeks of

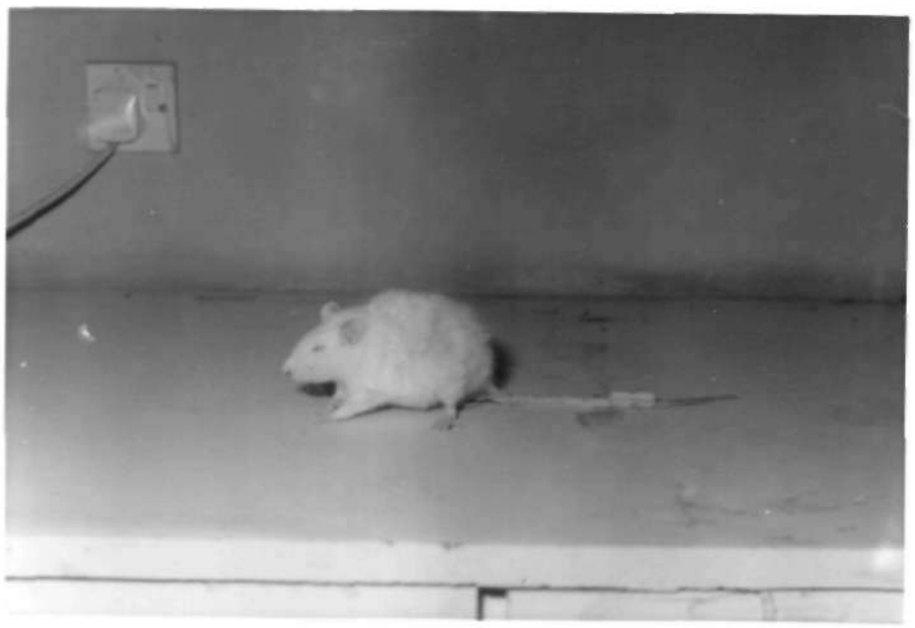
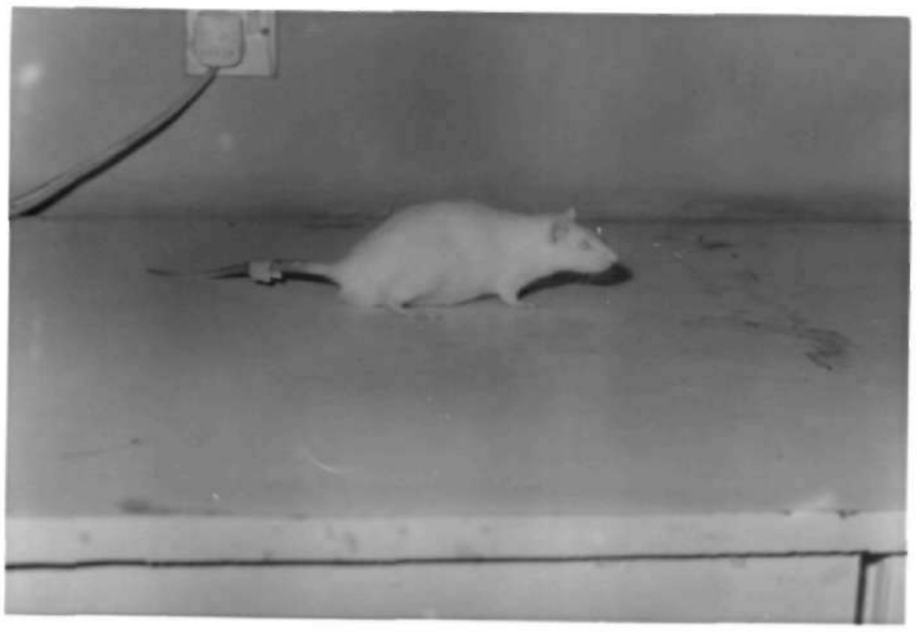


Figure 3

Alopecia observed in Rat treated with an acute dose of 400mg/kg TBTA.



The Albino Rat

being dosed with TPTA unlike the case of TBTA in which the surviving animals recovered completely only after a time period of 4 weeks.

3.1.3 GROSS AND HISTOPATHOLOGICAL FINDINGS

Tri-n-butyltin Acetate

250mg/kg: Four out of the 10 rats died. A gross post mortem examination of the rats revealed congestion of the brain, heart and lungs. This was observed in most of these dead rats. Petechial haemorrhages as well as empty stomach and intestine (sign of starvation) were seen in one of the rats in addition to the above lesions. Also two of the rats showed an additional catarrhal enteritis.

Histopathologic examination revealed that four dead rats had severe congestion and haemaorrhages in the lungs and brain (Figures 4 and 5). Mild congestion of the liver, petechial haemorrhage in the kidney as well as destruction of intestinal mucosa (Figure 6). No lesions were observed in the heart.

400mg/kg: At necropsy 4 out of the 7 dead rats showed congestion of the brain, heart and liver as well as catarrhal enteritis. Two of the rats showed haemorrhages in the brain and intestine and cystitis in addition to the above lesions. One rat also had congestion of the kidney tissues. In two of the rats empty stomach and intestine was observed, probably indicating lack of food intake during the period of observation.

The histopathological observations were similar to those of necropsy. All the major organs showed severe congestion and haemorrhage. For the rats which received lower dose (250mg/kg) of the drugs, severe pathologic effects were only observed in two organs namely, the lungs and the brain. In addition to the severe congestion in the organs, the lung tissue also revealed interstitial pneumonia and thickening of the alveoli septae in one of the rats. There was also complete necrosis of the intestinal villi.

Triphenyltin Acetate (TPTA)

350mg/kg: Two out of the ten rats died. At necropsy, both rats showed congestion of the brain and heart tissues, as well as haemorrhages in the lungs. One of the rats also showed congestion and petechial haemorrhages in the intestine and liver tissues respectively, in addition to the described lesions. One rat could not be posted.

Histopathologic examinations showed no lesion in the heart and spleen. However, severe congestion and haemorrhages were observed in the lungs of both rats. While the kidney, livers and brain showed only slight congestion.

500mg/kg: 6 out of the 10 rats died within 24hrs. At post mortem, congestion of the brain as well as petechial and generalised haemorrhages of the liver andlungs respectively, were observed. Five rats showed congestion of the liver, while two others showed catarrhal eneteritis. The intestines were empty in 2 rats and these ones had no enteritis.

A moderate congestion of the liver (Figure 7), brain and kidney (Figure 8) was observed in the tissue slides of all the 6 dead rats. However, petechial haemorrhages were seen only in the tissueslides of the kidneys. Also, as in the two dead rats dosed with 350mg/kg, there was severe congestion and generalised haemorrhage in the lungs. There was also exfoliation of the stomach/intestinal mucosa. The picture is somewhat similar but more severe with that of the 350mg/kg dosed rats.

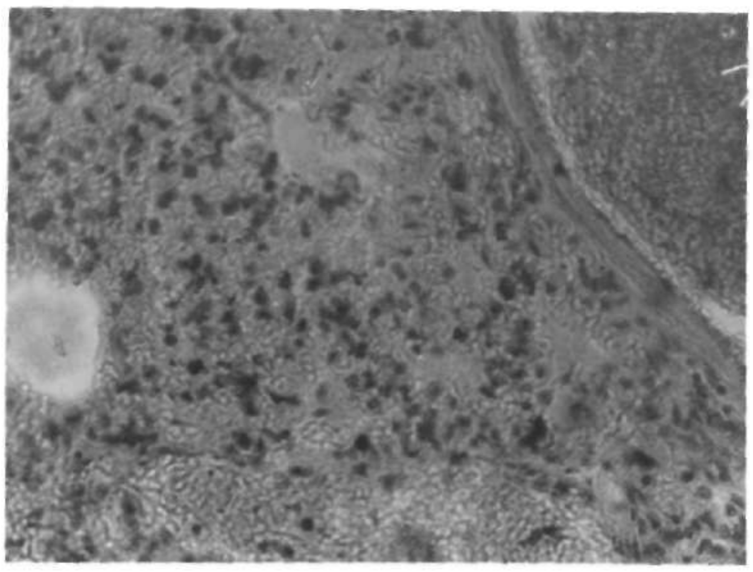


Figure 4
Part of the lung showing severe congestion in rat pretreated with an acute dose of 250mg/kg TBTA. x400

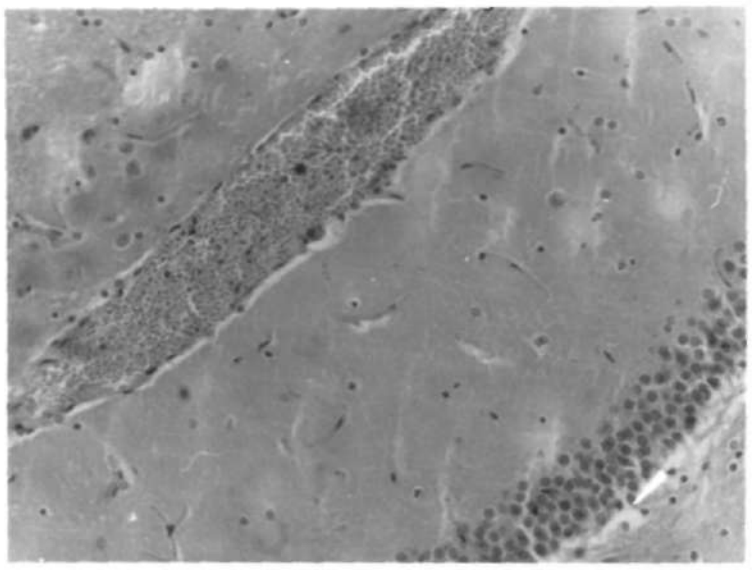


Figure 5
Part of the brain showing severe congestion in rat exposed to an acute dose of 250mg/kg TBTA. x250

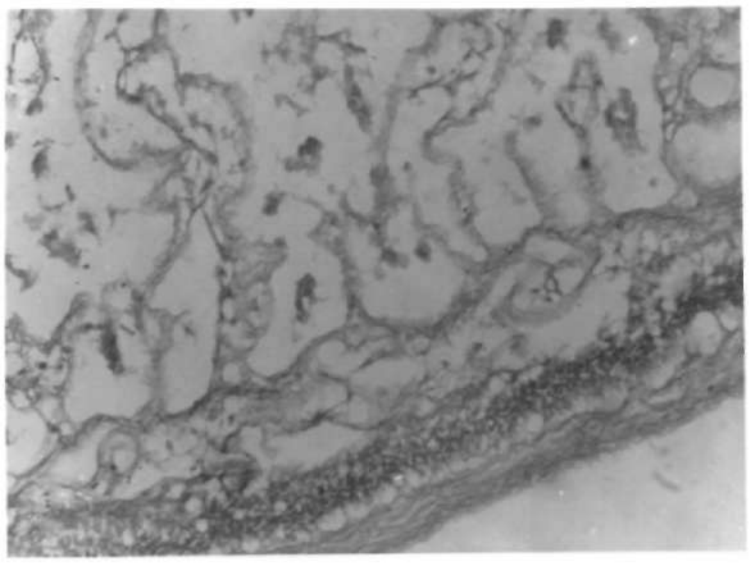


Figure 6
Severe destreuction of intestinal mucosa in rat exposed to 250mg/kg TBTA. x250

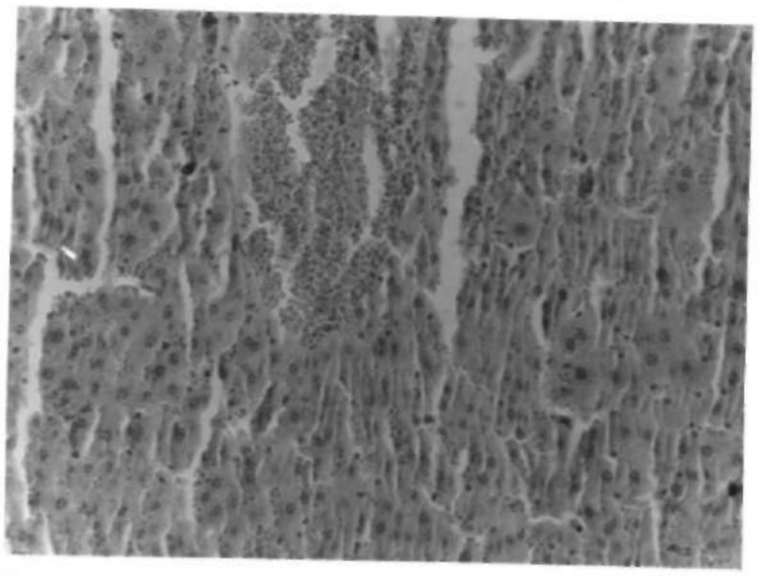


Figure 7
Moderate congestion of the liver in rat exposed to an acute dose of 500mg/kg TPTA. x250

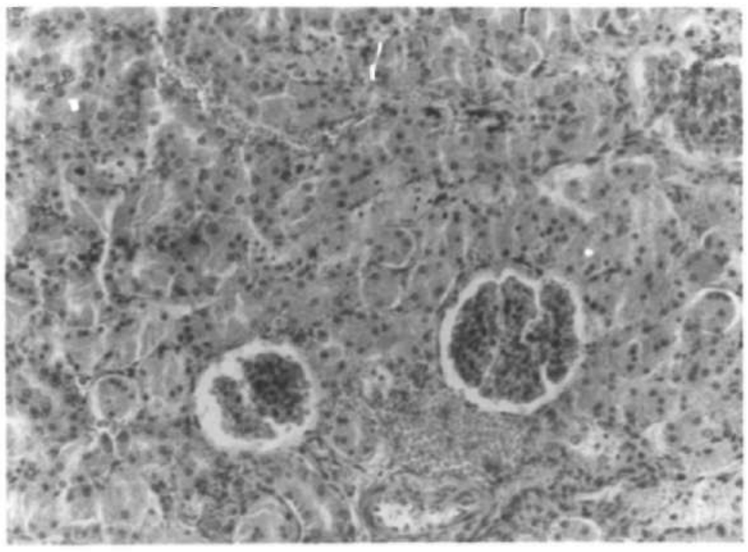


Figure 8

Part of the kidney tissue showing moderate congestion in rat treated with an acute dose of 500mg/kg TPTA. x 250

3.2 SUB-CHRONIC TOXICITY STUDIES

3.2.1 CLINICAL SYMPTOMS AND SIGNS

Tri-n-butyltin Acetate (TBTA)

Excitenment and dermal irritation characterised by excessive scratching of the mouth and nose were always observed within one hour of injection of the highest and middle doses (16 mg/kg and 8mg/kg) of TBTA respectively. These were usually followed by mild sedation - especially in the highest dose group. The lowest dose and control groups did not show these symptoms and the symptoms disappeared within 24 hours.

However, towards the end of the 3rd week some rats in both the highest and middle dose groups started having persistent sedation, followed by a prolonged shallow breathing which usually ended in death. 5 and 3 rats from the highest and middle dose groups respectivaly died after the 3rd week.

Triphenyltin Acetate (TPTA)

Very mild irritation was observed in the highest dose group, but aggressive behaviour was always observed few hours after the injection of the drug. Laboured breathing was also seen as in TBTA, but this was more pronounced in the last week of the expriment. 3 and 4 rats from the highest and middle dose groups died. The death was mainly due to injuries sustained from the fighting with members of the group in the case of the middle dose group.

3.2.2 NECROPSY FINDINGS

3.2.2.1 Organ/body Weight ratios

Table V shows the result of organ/body weight percentage ratios as determined for both treated and control rat groups. There were significant decreases only in the cases of lungs, kidneys and spleens of some treated groups (marked by asterix on the table V). For lungs, there was also a significant decrease in the organ/body weight percentage ratio for the groups of rats treated with 16 and 8 mg/kg (highest and middle doses, respectively) TBTA as well as the highest dose of TPTA (20 mg/kg). And for kidney the decrease is significant in rats treated with the highest and middle doses of TPTA (20 mg/kg and 10 mg/kg respectively).

There was significant decrease in spleen/body weight ratio only in the highest dose group for TBTA and TPTA. The t-values are highest in the case of the highest dose levels, signifying more decrease in the organ/body weight ratio percent.

Table V

mean organ/body weight percent ratio of rats exposed to TBTA at 16, 8 and 4mg/kg in groups A, B and C respectively, and TPTA at 20, 10 and 5mg/kg in groups D, E and F respectively over five weeks

	WEIGHT OF ORGANS ± SD					
Group	KIDNEY	LUNGS	LIVER	BRAIN	HEART	SPLEEN
A	0,8475 ±0,0475	0,8012* ±0,1178	3,429 ±0,344	0,8023 ±0,126	0,3160 ±0,0541	0,2687* ±0,0438
B	0,8474 ±0,1996	0,9084* ±0,0981	3,5084 ±0,293	0,8064 ±0,219	0,3734 ±0,067	0,4367 ±0,093
C	0,8900 ±0,1277	0,935 ±0,219	3,7618 ±0,2791	0,7992 ±0,146	0,3850 ±0,0829	0,594 ±0,056
D	0,726* ±0,0276	1,0519* ±0,374	3,4413 ±0,692	0,7908 ±0,792	0,3196 ±0,0789	0,3313* ±0,0647
E	0,7894* ±0,0641	1,0563 ±0,1475	3,504 ±0,2141	0,7858 ±0,066	0,3427 ±0,0586	0,5596 ±0,375
F	0,8961 ±0,0752	1,0710 ±0,1667	3,8858 ±0,3410	0,8146 ±0,0666	0,3229 ±0,0323	0,590 ±0,0838
G	0,9471 ±0,099	1,1128 ±0,0561	3,757 ±0,347	0,8534 ±0,1171	0,3482 ±0,0517	0,5963 ±0,056

G = control

* = significant decrease

3.2.2.2 Gross Pathological Findings

Tri-n-butyltin Acetate (TBTA)

Lungs, liver and intestines were the only organs found to have lesions in the pretreated groups. No gross lesion was observed in any of the control rats.

Three rats pretreated with 16mg/kg TBTA had pneumonia, congestion and haemorrhage in the lungs, and two others had petechial haemorrhage in the liver and intestine. Also 3 rats on 8mg/kg had petechial haemorrhage in the lungs, while two from the lowest dose (4mg/kg) group had haemorrhagic liver only.

Triphenyltin Acetate (TPTA)

On visual examination of the organs of the pretreated groups, only lungs and gastrointetinal tract were shown to have lesions. Most of the rats in the highest dose (20mg/kg) group had petechial harmorrhage in the lungs. 3 rats from 10mg/kg dose group had congestion, haemorrhage and abscess of the lungs respectively. Two of these also had haemorrhagic intestine. Only two rats from the lowest dose (5mg/kg) group had haemorrhagic lungs.

3.2.2.3 Histopathological Findings

Tri-n-butyltin Acetate (TBTA)

Group 1 (16mg/kg): Most rats in this groups had congestion and haemorrhages as the main histopathological lesion.the lesion appear to be dose related as it is more severe in those rats that survive up to the end of the experiment. It was in few cases accompanied by necrosis and interstitial preumonia with infiltration of macrophages (Figure 9), as well as emphysema (Figure 10) which indicate laboured respiration that was observed as the major symptom of TBTA toxicity in this study. The liver was the next organ in order of the occurance and of severity of damage; up to 5 rats in this group showed mild to severe congestion (Figure 11). One rat had slight congestion in the heart, as well as congestion and thickening of the blood vessels of the spleen. Another rat had congestion of the brain and mild congestion of the kidney. Also in most of the rats there was massive destruction of the intestinal mucosa. None of the control rats had any of these lesions under histopathological examination.

Group 2 (8mg/kg): Three rats in this group had moderate congestion in the lungs, only one had congestion together with haemorrhage and infiltration of lymphocytes and neutrophils (Figure 12). This is another indication of dose dependency of lung lesion as regards to TBTA. As in group 1 above liver is the next organ in order of severity of lesion, up to 3 rats had congestion. Two rats had exfoliation of the intestinal mucosa. No other organs apart from the ones mentioned above showed any lesion in this group.

Group 3 (4mg/kg): only 2 rats had areas of petechial haemorrhage in their lungs and one had expoliation of intestinal wall. No lesion was observed in other organs.

Group 4 (control): only one rat had mild destruction on mucous membrane in the stomach. One control group was used for both TBTA and TPTA.

Triphenyltin Acetate (TPTA)

Group 1 (20mg/kg): Histopathological examination indicate mild to severe lesions in the lungs, intestine and kidneys as well as in the brain. No lesion was observed in the liver as compared to that seen in the highest sub-chronic dose of TBTA (16 mg/kg above). Also other organs were not affected.

Most rats in this group had mild to severe congestion of the lungs. This was accompanied by massive infiltration of lymphocytes around the bronchioles in 2 rats. Three rats had severe exfoliation of mucosa, 2 had severe nephrosis (figure 13), and another 2 had mild congestion in the brain in addition to the congestion of the lungs.

Group 2 (10mg/kg): Congestion and petechial haemorrhage of the lungs as well as kidney nephrosis were seen in 3 rats of this group. In addition there was mild haemorrhage in the brain and destruction of intestinal mucosa in 1 and 2 rats of this group respectively.

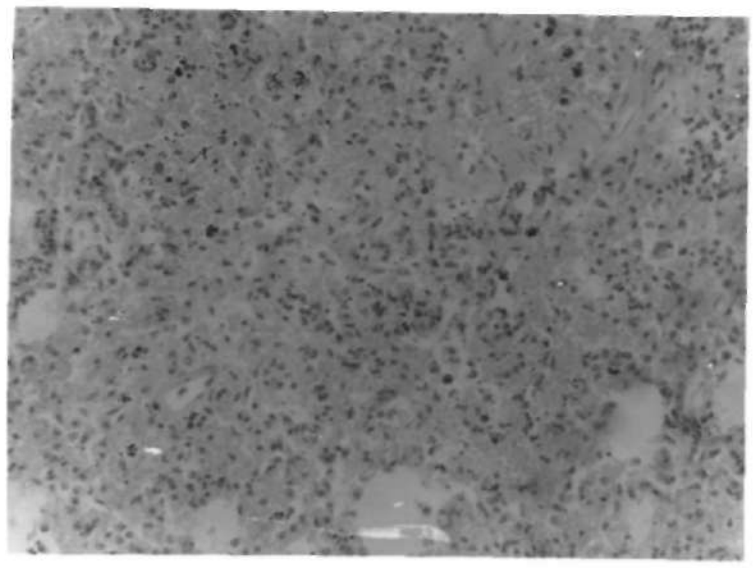


Figure 9
Part of the lungs showing severe congestion and interstitial pneumonia in rat exposed to sub-chronic doses of 16mg/kg TBTA. x 400

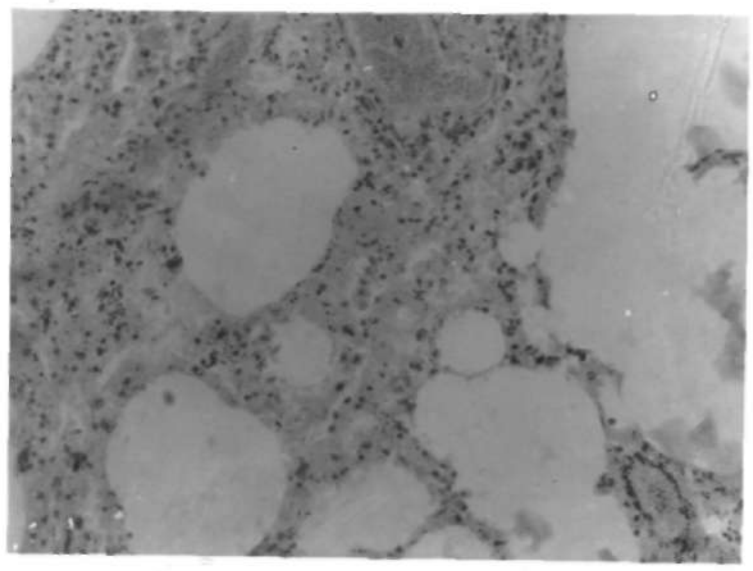


Figure 10
Part of the lung showing emphysema and severe congestion in rat treated with sub-chronic doses of 16mg/kg TBTA. x 250

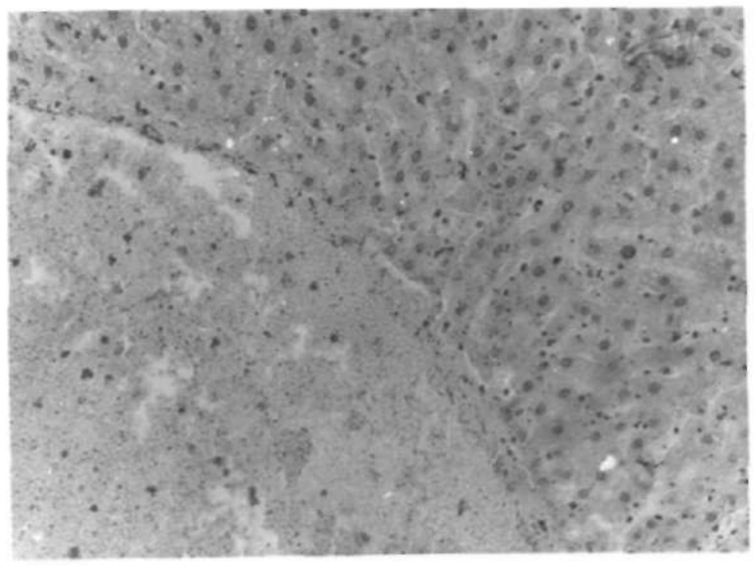


Figure 11
Liver tissue showing severe congestion in rat exposed to sub-chronic doses of 16mg/kg TBTA. x 250

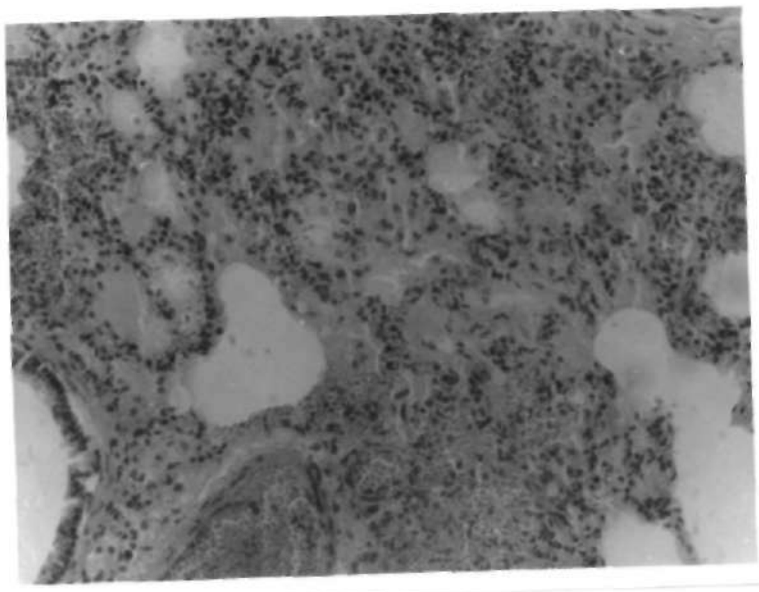


Figure 12
Part of the lungs showing massive proliferation of lymphocytes and neutrophils in rat exposed to 8mg/kg TBTA for 5weeks. x 250

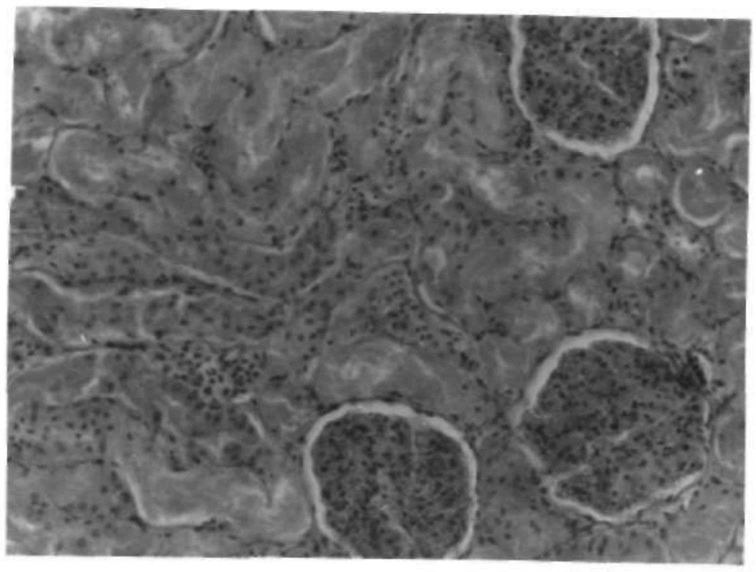


Figure 13
Part of the kidney showing severe nephrosis in rat exposed to 20mg/kg TPTA for 5weeks. x 250

Group 3 (5mg/kg): One rat had suppurative pheumonia and mild disruption of mucosa membrane of the intestine.

3.2.3 HAEMATOLOGY FINDING

Mean blood values obtained from both pretreated and control groups are shown on table VI. There is no significant difference in the Red Blood Cell (RCB) count and Haematocrit (Hct) values between the treated and the control groups, and all values are within the normal range.

The values for total leucocyte (WBC) count were also within the normal range of 5,000-25,000/mm^3 reported for rats (Schemer, 1958). Even though the values in all treated groups differ from the control group, the difference is not significant.

There is no significant change in the neutrophil (Neut) count between all the treated groups and the control group.

Only group D (20mg/kg TPTA) and E (10mg/kg TPTA) showed significant decrease in the mean lymphocytes (Lymh) count compared to the control group.

In the case of monocyte (Mono) there is significant decrease only between group D (20mg/kg TPTA) and the control. All other groups show no significant difference with the control. For eosinophils (Eos) only group D and E differed with the control (Table VI).

Table VI

Mean blood values of rats exposed to TBTA at 16, 8 and 4mg/kg in groups A, B and C respectively and TPTA at 20, 10 and 5mg/kg in groups D, E and F respectively over five weeks.

BLOOD VALUES ± S.D.							
Group	Hct %	RBC M/mm^3	WBC $1000/mm^3$	Neut %	Lymp %	Eos %	Mono %
A	40,333 ±7,318	8,250 ±0,178	6,066,667 ± 865,384	26,00 ±27,118	74,333 ±3,210	0,333 ±0,471	0,667 ±0,943
B	41,429 ±3,289	8,221 ±0,775	6,214,286 ± 842,494	26,86 ± 4,324	74,300 ±2,828	0,286 ±0,452	0,443 ±0,350
C	40,80 ±5,724	8,275 ±1,096	5,930,00 ± 885,494	27,60 ± 4,716	78,00 ±7,183	0,200 ±0,400	0,400 ±0,663
D	41,00 ±1,927	8,164 ±0,579	5,885,714 ± 762,381	27,143 ± 3,523	71,143 ±4,549	0,714 ±0,881	1,857 ±1,457
E	42,167 ±2,267	8,383 ±0,358	6,200,00 ± 687,992	29,667 ± 3,496	68,333 ±5,121	0,667 ±0,943	0,500 ±0,764
F	40,00 ±3,715	8,05 ±0,648	5,710,00 ± 704,90	26,90 ± 4,085	78,40 ±4,086	0,300 ±0,640	0,400 ±0,663
G	40,375 ±2,781	8,056 ±0,896	5,728,00 ± 145,37	26,75 ± 3,491	78,50 ±4,25	0,25 ±0,433	0,75 ±0,829

G = Control group

CHAPTER FOUR

DISCUSSION

4.1 Acute Toxicity

The result of acute toxicity test with TBTA and TPTA (section 3.1) showed a moderate acute oral toxicity for these compounds in rat. The difference between the LD_{50} for TBTA (297.54mg/kg) and TPTA (402.38mg/kg) as stated earlier (section 1.6.1) could be due to the nature of the organic groups attached to the tin atom (Neumann, 1967). The lower the group the higher the toxicity, hence in this case the butyl group in TBTA is expected to confer higher toxicity than the phenyl group found in TPTA.

There is wide variation in the LD_{50} figures reported in the literature for both compounds, even though most reports show the compounds to posses intermediate and specie dependent toxicity. In rat studies oral LD_{50} of 380.2mg/kg (Neumann, 1967), 380mg/kg (Poller, 1970) and 287mg/kg (Esievo, 1984) were obtained for TBTA. And for TPTA 125-150mg/kg (Poller, 1970), 491mg/kg (Stonner, 1966) and 340mg/kg (Lodhi, 1985) were also reported. The LD_{50} in this study therefore, is comparable to some of the reported LD_{50} values. Both Esievo (1984) and Lodhi (1985) used the same chemical source with the present study and probably the same specie of rats, hence the slight difference between our result and theirs could be due to differences in batches used. It was discovered during the course of our pilot testing that two different batches of TBTA in particular differed in severity of effects. This necessitated the use of one batch for all the studies. However, previous studies have not reported any differences in LD_{50} values due to differences in chemical shelf life.

In this study as in that of Esievo (1984) and Lodhi (1985) the signs and symptoms of TBTA and TPTA poisoning indicate neurotoxicity and the involvement of the respiratory center. The symptoms are quite similar in both TBTA and TPTA. However, in TBTA they are more severe. Also the severity is more at higher doses.

The similarity in symptoms could suggest similarity in mechanism of action for TBTA and TPTA. Aldridge (1976) attributed the mechanism of toxicity of all trioganotin compounds to the derangement of the mitochondrial function which also result into the uncoupling of oxidative phosphorylation. Stock *et al.* (1970) showed that the order of effectiveness for triorganotin compounds in inhibiting coupled respiration was Tributyl > Tripropyl > Triphenyl > trimethyl. Earlier, Moore and Broody (1961) have suggested the inhibition of the hydrolysis of adenosine triphosphate (ATP) and uncoupling of oxidative phosphorylation taking place in the mitochondria to be a general cellular mechanism of tin toxicity. Symptoms like loss of appetite might be secondary to the respiratory effects. Skin irritation could be due to rapid absorption by the skin in case of TBTA. TPTA is said to be poorly absorbed by the dermis (section 1.5.1)

Congestion, haemorrhages, and necrosis are the main lesions observed in various organs. Congestion in the lungs is more pronounced, especially at higher dose of both TBTA and TPTA. The lesions suggest a direct effect of the chemicals as evidenced by the presence of cellular infiltration which serves to clear the debris of dead tissues. It could also be secondary to the uncoupling action of TBTA and TPTA which result in the shortage of ATP in all tissues and organs of the body. The consequence of this is the inhibition of all cellular functions including respiration and subsequent death of tissue or the animal itself.

4.2 Sub-Chronic Toxicity

The result of sub-chronic toxicity test in rats for this experiment indicate that doses up to 4mg/kg TBTA and 5mg/kg TPTA could not be used directly in mammals up to 5weeks without any adverse effects. However, they may be okay if incorporated in food. Moreover the minimum inhibitory concentrations (MIC) obtained against the specie of fungi that cause yam rot is far below these levels for both TBTA and TPTA (Olunrinola *et al.*, 1988).

In this result as little as 8mg/kg TBTA and 10mg/kg TPTA resulted in the death of some of the experimental animals. This indicate a low lethal value for sub-chronic test. However, for organotins sub-acute and chronic toxicity levels are observed to be far less than the acute toxicity values. Mazaev and Korolev (1969) reported death at a level of 50mg/kg TBTA when the drug was given to rats in the diet for 17 days. Klimmer (1964) recorded loss of appetite, weight gain and death in dogs at a level of 12mg/kg with cyclohexyltin hydroxide given in the diet. Moreover some of the recorded death in our study could also be attributed to infection, because some rats died as a result of injuries sustained from their partners. The presence of infiltration in some rats' tissue (section 3.2.2.3) seem to confirm this.

The symptoms observed during sub-chronic exposure with the chemicals show reversibity and a good correlation with dose. However, there was no good correlation with the lesions observed especially in case of TBTA.

The results of the gross and histopathologic examinations of the sub-chronic study in rats revealed the presence of lesions in many organs. The observed lesions were in some instances accompanied with cellular infiltration and haemaorrhages, depending on the given dose of administered toxicant.

Histopathological examination shows the lesions to be more extensive in the lungs, liver, intestine and kidney. The significant reduction in reported organ/body ratios (Table V) for lungs and kidney of higher dose groups serve to comfirm the severity in these organs. In the litterature sub-chronic doses of organotin were shown to produce many lesions. (see introduction) The mechanism of formation of these lesion might be related to be effects of the TBTA and TPTA on the production and breakdown of the ATP in the mitochondria mentioned earlier in the discussion.

4.3 Haematological Findings.

The mean values of haematology parameters obtained in this study did not differ from the normal range for rats (Schemer, 1958). However, some significat differences were obtained between the control and treated groups in some parameters.

There was significant decrease in the mean lymphocytes count for 20 and 10mg/kg TPTA-treated groups. A reduction in the number of lymphocytes and leucocytes were recorded in female and male guinea pigs on dietary level of 5 and 10mg/kg TPTA, respectively in a 12 weeks study (WHO/FAO 1971). Monocytes and eosinophil counts also differ from the control for the two groups mentioned above (Table VI). These differences in case of the rat group treated with 10mg/kg TPTA might have arisen from the infections that developed in few of them as a result of injuries they sustained. Since some of these cells are associated with infections. Godwin, *et al.* (1964) found an association between disease and changes in some blood parameters. He reported high values for WBC, neutrophil and monocytes and lower values for lymphocytes in diseased rats as compared to the healthy ones.

4.4 Conclusion

If sufficient precaution is taken in application, and moderate doses are used, the residual levels which one would encounter in yam seedling pre-treatment with TPTA and TBTA or in direct yam preservation treatments would seem unlikely to provoke incidences of human toxicosis. However, it has been shown especially for chemicals used in the treatment of food crops that an agent may interact with other compounds to which the crop is concurrently exposed to like pesticides for instance or with compounds contained in other food items consumed at the same time; or common therapeutic agents (see Iyaniwura, 1988). Thus evaluation of the toxicity of these compounds in future should give consideration to the possibility of interaction with other potentially toxic agents that may augment or modify their respective toxicities. These should be allowed for in setting dietary limits for these two copounds.

REFERENCES

Adaudi, E. L. (1983)

Fungicidal Properties of organotin comppounds on yam rot Fungi.

Department of Chemistry A. B. U. Zaria Project work

Aldridge, W. N. (1976)

The Influence of organotin compounds on mitochondrial functions In: Zuckerman J. J. (ed) Organotin compounds, New chemistry and applications, Washington, D. C. American Chemical Society, pp. 186 - 196.

Anderson, Herbert H. and Vasta Joseph (1950)

American J. org. Chemistry, 19 : 1300.

Akagi, H. and Sakagami, Y. (1971)

Degradation of organotin compounds by ultraviolet ray.

Japanese Rep. Public Health Inst., 20 : 1-4.

Aliu, Y. O. and Nwude, N. (eds) (1982)

Veterinary Pharmacology and Toxicology Experiments. Baraka Press Zaria Page 104 - 112

Anne-Marie, C. (1984)
Organotin compounds used in Agriculture, In : Tin and it's uses, № 140 : 12 - 13

Anon, (1967)
Chem-Eng. News (52), 45, 24. In : Present Trends and Perspectives in Organotin Chemistry and Industry, 1920 : 644 - 646.

Barnes, J. M. and Magee (1958)
Lesion produced experimentally by dibutyltin salt
J. Pathol. Bacteriol. 75 : 267 - 279

Barnes, J. M. and Stone, H. B. (1958)
Toxic Properties of some dialkyl and trialkyltin salts.
British Journal of Indian Medicine 15 : 15-22

Barnes, R. D. Bull A. T., and Poller, R. C. (1973)
Studies on the persistence of the organotin *fungicide, fentin* acetate (triphenyltin acetate) in the soil and on surfaces exposed to light.
Pestic - Sci., 4 : 305 - 317.

Bassinadale, A. R. Earborn, C., Tylor, R.
Thompson, A. R., Walton D. R. M., Cretnay, J. and Wright, G. J. (1971)
Aromatic reactivity 46. Interpretation of substituent effects in base - catalyzed clearage of aryltrimithylsilanes and aryltrimethyl stannanes.
J. Chem. Soc. B., 1155 - 1161

Bonire, J. J. (1977).
Synthetic, Spectral and Fungicidal Aspects of some Organotin Compounds.
Department of Chemistry, A. B. U. Zaria,
M. Sc. Project Work.

Bridges, J. W. Davies, D. S. and Williams, R. T.
(1967) The fate of Ethyltin and diethyltin derivatives in the rat.
Biochem. J., 105 : 1261 - 1266

Bruggemann, J. and Barth, K. and Niesan K. H. (1964a) [Experimental studies on the content of triphenyltin acetate residues in turnip leaves, ensilage of turnip leaves and in excrements of animals fed with these products] Zentralbl. Vet. Med. A, 11 : 4 - 19 As reported in WHO health criteria Nº 15 (1980) page 43.

Bruggemann, J. and Klimmer D. R. (1964). [Nutritional Physiological, analytical and toxicogical Studies on fungicidal triphenyltin acetate]. Zentralbl - Vet. Med. 17, 11 : 1 - 3. As reported in WHO health criteria 15 (1980) page 43.

Bruggemann, J., Klimmer, O. R. and Niesar, K. H. (1964b) [The importance of triphenyltin acetate residues in plants and animals from a hyganic toxicologic standpoint]. Zentralbl. Vet. Med. A, 11 : 40 - 48. As reported in WHO Health Criteria 15 (1980) page 43.

Canjolle, E., Lesbre, M. and Mayniev, D. (1954)
Surla toxicite du tetramethyl stannane et du tetraethyl stannane C. R.
Acad - Sci (Paris) 239 : 556 - 558
As reported in WHO Health Criteria 15 (1980) p. 75.

Cenci, P. and Cremonini, B. (1969)
[Thin layer chromatography of two organotin pesticides and their behavior in various types of soils].
Ind. Sac. Hal. 69 : 313 - 316. As reported in WHO Health Criteria 15 (1980) p. 43.

Chapman , A. H. and Price, J. W. (1972)
Degradation of triphenyltin acetate by ultraviolet light.
Int. Pesticide control, 14 : 11 - 12.

Coles, E. H. (1974)
Veterinary Clinical Pathology 2nd Edition. Page 615 - W. B. Sanders Company Philedelphia, Conlter Electronics Inc. Hialech, Florida.

Cremer, J. E. (1958)

The Biochemistry of organotin compounds : The comversion of tetraethyltin into triethyltin in mammals.

Biochem. J., 68 : 685 - 692.

Cremer, J. E. (1957)

The metabolism *in vitro* of tissue slices from rats given triethyltin compounds.

Biochem. J., 67 : 87 - 96.

Esievo, V. O. (1984)

Preliminary Anatomical Profile and Toxicological studies of Tri-n-butyltin Acetate.

Department of Pharmacology and Clinical Pharmacy, A. B. U., Zaria, B.Sc. Project Work.

F. A. O./W. H. O. (1971)

Evaluations of some pesticide residues in food. Rome, Food and Agricultural Organization of the United Nations/World Health Organization pages 327 - 547.

Fish, R. H., Kimmel, E. C. and Casida, J. E. (1975) Bioorganotin chemistry. Biological oxidation of tributyltin derivatives. J. Organometal Chem. 93(1) : C_1-C_4.

Freitag, K. D. and Bock, R. (1974b) Degradation of triphenyltin chloride on sugar beet plants. Pesticide Sci., 5 : 731 - 739.

Gaines, T. B. and Kimbrough, R. D. (1968) Toxicity of fentin hydroxide to rats. Toxicol. Appl. Pharmacol., 12 : 397 - 403.

Gaunt, I. F., Collery, J., Grasso, P. and Creasy, M. (1968) Acute and short term toxicity studies on di-n-butyltin chloride in rats. Food Cosmet. Toxicol. 6 : 599 - 608.

Godwin, K. O., Fraser F. J. and Ibbotson R. N. (1964) Haematological observations on healthy (SPF) rats. Brit. J. Exp. Path. 45, 514 - 24

Goodrich Company, B. F. U. S. 3, 426, 473 (1969)
As reported in Vander Kerk, J. G. M. 1970, Present Trends on Perspectives in Organotin Chemistry page 646.

Guardas Cione, V. and Di Bosco, M. M. (1967)
[Contribution to the knowledge of occupational disease due to pesticides; Three cases of acute intoxication by triphenyltin acetate fungicides]. Lav. Um., 19 ; 307 - 313 As reported in W.H.O., Health Criteria 15, (1980).

Hartel, K. (1958)
Angew-Chem. 70 : 135.

Heath, D. F. (1963)
Some problems in the determination of residues in plants and animals. In : Proceedings of a symposium, Radiation and Radioisotopes Applied to Insects of Agricultural importance, Athens, 22 - 26 April 1963, Vienna, International Atomic Energy Agency, p 185 - 193.

Heath, D. F. (1967)

The retention of triphenyltin and dieldrin and its relevance to the toxic effects of multidosing, In : Radioisotopic detection of Pesticide Residues, Proceedings of a Panel, Vienna, 1965 page 18 - 26 (Abstracted in: Genev. Chem. Abstr., 67 : 1828 V).

Hedges, T. R. and Zaren, H. A. (1969)

Experimental papillodema: A study of cats and monkeys intoxicated with triethyltin acetate. Neurolgy, (Minneapolis), 19 : 359 - 365.

Herok, J. and Gotte, H. (1964)

[Radiometric Study on the metabolism of triphenyltin acetate in milk of sheep]. Zentralbl. Vet. Med. A., 11 : 20 - 28. As reported in W.H.O. Health Criteria 15 (1980)

Horacek, V. and Demcik (1970)

Group poisoning in spraying of field cultures with Brestan 60 triphenyltin acetate] Pract. Lek., 22 : 61 - 66. As reported in W.H.O. Health Criteria 15 (1980).

Ignatjeva, M. A., Kuznetsov, I. G., Mirskov, R. G. and Vilasov, V. M. (1968) [Toxicology of acetylene organotin compounds of triethylstannyl range.] 12V. Sib. Otdel. Akad. Nauk SSSR, 1(5): 113 - 119. As reported in W.H.O. Health Criteria (1980) page 146.

Iyaniwura, T. T. (1988)
Pharmacological interactions and combined toxicity of exposure to environmental chemicals.
Reviews in Environmental Health, in press.

Kimmel, E. C., Fish, R. H. and Casida, J. E. (1977) Biorganotin chemistry: Metabolism of organotin compounds in microsomal monooxygenase systems and mammals.
J. Agric. Food Chem., 25(1) : 1-8.

Klimmer, O. R. and Nebel, I. U. (1960)
Arzneimittel - Farsh 10, 44. As reported in WHO Health criteria № 15 1980.

Klimmer, O. R. (1963)
[Triphenyltin acetate and the toxicology of organic tin compounds]
Arzneittel - Farsh 13 : 432 - 436
As reported in WHO Healh Critria, 15, (1980)

Klimmer O. R. (1964)
Toxicological studies on triphenyltin acetate
Zentralbl. Vet. Med., R. A. 11 : 29 - 39 : In WHO, Health Criteria 15, 1988 pp 69.

Lodhi, F. (1985)
Preliminary Antimicrobial Profile and Toxicological studies of Tri-phenyltin Acetate.
Department of Pharmacology and Clinical Pharmacy A. B. U. Zaria. B. Sc. Project.

Marks, M. J., Winek, C. L. and Shanor, S. P. (1969) Toxicology of Triphenyltin hydroxide..
Toxicol. Appl. Pharmacol., 14 : 627.

Mazaev, V. T. Golovanov, O. V. Igumov, A. S. and Tsay, V. V. (1976) [Problem of the Transformation of organotin compounds in a water medium]
Gig I Sanit,3 : 17 - 20 (Russian): As reported in WHO, Health Criteria 15, 1980 pp. 43 - 45.

Mazaev, V. T. Korolev, A. A. (1969)
[Experimental standardiztion of the maximum permissible concentration of the dichlorbutyltin in surface water].
Prom Zagrjarnenija Valoemor, № 9 pp 15 - 37 (Russia). In WHO Health Criteria 18 : 1980 pp. 72.

Mazaev, V. T. and Slepina, T. G. (1973)
[Experimental data on hygienic standardization of dibutyltin sulfide in water bodies].
Gig I. Sanit., 8 : 10 - 13 (In Russian) : In WHO Health Criteria 15 : 1980 pp 74.

Mijatovic, M. (1972)
[Chronic hepatitis due to exposure to Brestan].
Jugosl. Inostr. Dox. Zast Radu, 6 : 3-9 In WHO Health Criteria 15 p. 75

Miller,L. C. and Tainer. M. L. (1944)
Estimation of the ED50 and its Error by means of Logarithmic - Probit Graph. Proc. Soc. Exp. Biol. Med, N. Y. 57 : 261 - 264.

Moore, K. E. and Brody, T. M. (1961)
Effects of triethyltin on mitochondrial swelling.
Biochem. Pharmacol, 6 : 134 - 42.

Neuman. P. W. (1967)
The organic chemistry of Tin p.232.
London Willy

Noltes, J. G. Inijten, J. G. A. and Vander Kerk, (1961)
J. Appl. Chem., 11, 38.

Olurinola, P. F., Ehinmmidu, J. O. and Bonire, J. J. (1988) Activity of TBTA on yam rot isolates, unpublished

Pelikan, Z and Cernny, E. (1968)
The toxic effects of tri-n-butyltin compounds on white mice.
Arch. Toxicol. 23 : 283 - 292

Pelikan, Z. and Cernny, E. (1968)

The effect of low doses of bis-(Tri-n-butyltin) Oxide on the skin of rats.

Brufsdermatosan, 16 : 340 - 349.

In WHO Health Criteria 15 : 1980.

Pelikan, Z., and Cerny, E. 1967)

The effect bis (tri-n-butyltin) oxide (TBTO) on the skin of rats

Berufsdematosan, 17 : 305 - 316.

In WHO Criteria 15 : 1980.

Pelikan, Z. and Cerny, E. (1970a)

Toxic effects of some mono-n-butyltin compounds on white mice.

Arch. Toxicol., 26 : 196 - 202.

Pelikan, Z. and Cerny, E. (1970b)

Toxic effects of some mono-n-butyltin compounds on white mice.

Arch. Toxicol, 27 : 79 - 84.

Poller, R. C. (1970)

Physical measurement of organotin compounds. The chemistry of organotin compounds P. 59 and 274.

Roberts, R. M. G. and EL - Kaissi, F. (1968)
Base Catalyzed cleavage of allylic derrivatives of group IV A elements.

Schemer S. (1958)
Die Blutmorphologie der Laboratoriumstiere, 2nd edn. pp 41 - 74 : Leipzig : J. A. Barth. In : Ernest Cotchin and Francis J. C. Roe (eds) Pathology of Laboratory Rats and Mice. Blackwell Scientific Publications Oxford and Edinbugh (1967) pp. 509.

Sheldon, A. W. (1975)
Effects of organotin antifoulin coatings on man and his environment.
J. Paint Technol, 47 : 54 - 58.

Shirasu, Y. (1970)
Acute and sub-chronic toxicology studies of Dawco 213 in Mice and Rats. Test report on the sub acute toxicity of an insecticide Dowco 213.
Tokyo, Animal Pharmacology Institute of Physical and Chemical Research, 11 p 35.

Sienen, W. Vos, S. G. Spanje, I.Van, Snock, M., Brands, R., and Hooykaas, H. (1977). Toxicity of organotin compounds: A comparative *in-vivo* and *in-vitro* studies with various organotin and organo lead compounds in different animal species with special emphasis on lymphocyte cytotoxicity. Toxicol. Appl. Pharmacol., 42 : 197 - 212.

Spector, W. S. (Ed.) (1955) Handbook of Toxicology, Carpenter Co., Springfield, Ohio.

Stockdale R. Dawson , A. P. and Schuyu, M. J. (1970) Effects of trialkyltin and triphenyltin compounds on mitochondrial respiration. Eur. J. Biochem., 15 : 347 - 351.

Stonner, H. B. (1966) Toxicity of Triphenyltin British J. Indian Medicine, 23 : 222 - 229.

Stonner, H. B. Barnes, J. M. and Duff, J. I. (1985) Studies on the toxicity of alkyltin compounds. British J. Pharmacol. 10 : 16 - 25.

Suzuki, K. (1971)
Some new observations in triethyltin intoxication of rats.
Exp. Neurol., 31 : 207 - 213.

Tamaru, Hirokuni (1965)
Chemical Abstract; 63 : 18959 c. h.

Vander Berghe, E. V. and Vander Kalen, G. P. (1966) Alkyl Redistribution Reactions of (CH₃nShX4-n compounds (X = CL, Br, I).
J. organometalic Chem. 6, 522.

Vander Kerk, J. G. M. and Luijtem, J. G. A. (1954)
Biocidal Property of organotin compounds.
J. Appl. Chem.**4**, P. 301 - 314.

Vander Kerk, G. J. M. (1970)
Present Trends and Perspectives in Organotin Chemistry.
Chemistry and Industry, 644 - 645.

Verschuuren, H. G. Ruitenberg, E. J., Peetoom, F., Helleman, P. W., and Ven Esch, G. J. (1970).
Influence of triphenyltin acetate on lymphatic tissue and immune response in guinea pigs.
Toxicol. Appl. Pharmacol., 16 : 400 - 410.

Vanke D. S. (1967)

Germ Pat. Appl. I, 269 - 292

As reported in Vander Kerk G. J. M. (1970) p.

45.

WHO Health criteria № 15 (1980) Pages 25, 39,

43, 61, 327, 332, 333, 337, 341.