AMITRAZ: A DEMOGRAPHIC SURVEY OF POISONING AT KENYATTA NATIONAL HOSPITAL AND ACUTE TOXICITY STUDY IN RATS

BY

TOM BOSIRE MENGE (B. PHARM)

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science in Pharmacology and Toxicology

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Department of Public Health, Pharmacology and Toxicology

Faculty of Veterinary Medicine

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University of Nairobi

2007
DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

Tom Bosire Menge

This thesis has been submitted for examination with our approval as University supervisors

Dr. Mbaria J.M. B.V.M., M.Sc., PhD.

Prof. Maitai C. K. B.Pharm, PhD.

Dr. Ogara W.O. B.V.M., M.Sc., PhD.
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I dedicate this work to my wife, Rose, and my children Sharon and Allan
ABSTRACT

Pesticide poisoning is an important cause of worldwide morbidity and mortality. This is because pesticides are widely available and extensively used in households for human and animal hygiene and in agriculture for crop protection and pest control. Amitraz is a synthetic formamidine pesticide that is used to control ticks, mites and other insects in livestock and other domestic animals, as well as red spider mites and other pests in fruit crops. It is widely available in Kenya where it is mainly used in cattle to control ticks and mites.

The current study was undertaken to determine the prevalence of amitraz poisoning among patients admitted with pesticide poisoning in Kenyatta National Hospital, Nairobi. The other objective was to determine the intraperitoneal median lethal dose (LD$_{50}$) of amitraz in laboratory animals and study the effect of yohimbine antagonism and urinary alkalination on the relative toxicity of amitraz.
A demographic survey was conducted to determine prevalence of amitraz poisoning in Kenyatta National hospital and document the diagnosis, management and outcome of all patients admitted with pesticide poisoning. Data was compiled by reviewing files from 1004 patients admitted at the hospital with poisoning for a six year study period from January 2000 to December 2005. Pesticide poisoning accounts for 0.2% of the patients admitted in the hospital. Amitraz accounted for 8.3% of the cases reviewed, while rodenticides accounted for 34.9% and organophosphates 34.0% of poisoning patients admitted in the hospital. The survey indicated that amitraz is assumed to be an organophosphate and the management of patients consistently made with this assumption. Poisoning occurs in all age groups, with children below 12 years accounting for 9% of the cases reviewed.

Acute toxicity studies were conducted to determine the median lethal dose (LD$_{50}$) of amitraz in rats, and the effect of yohimbine antagonism and urinary alkalination on the relative toxicity of amitraz. The intraperitoneal acute median
lethal dose (LD$_{50}$) and 95% confidence limits in mg/kg for amitraz in rats was 75.01 (58.1 to 96.8). The intraperitoneal LD$_{50}$ following yohimbine antagonism was 78.11 (65.84 to 92.66) mg/kg while the intraperitoneal LD$_{50}$ following urinary alkalization with sodium bicarbonate was 91.85 (72.86 to 115.8) mg/kg.

The study shows that yohimbine antagonism marginally, though significantly increases the acute median lethal dose, and hence reduces the relative toxicity of amitraz in rats. Urinary alkalization with sodium bicarbonate also increases the LD$_{50}$ and reduces the relative toxicity of amitraz in rats. As indicated, the management of most cases of amitraz poisoning is based on the assumption that amitraz is an organophosphate; the study therefore recommends for the education of doctors and others healthcare professions on the classification and management of amitraz and other common toxicants. Further research is necessary to determine the usefulness of urinary alkalization in the management of amitraz poisoning.
CHAPTER ONE:

INTRODUCTION

1.1 INTRODUCTION

Hospitals throughout the world handle a large number of individuals admitted with suspected poisoning. Toxicity due to drug and chemical exposure was described in the United States in the last decade as a national epidemic (Kulig, 1992). The range of substances encountered is huge and includes Pharmaceutical agents, illicit drugs, solvents, pesticides, toxic metals and a host of other industrial and environmental poisons.

The causes of poisoning are either accidental or deliberate. Childhood poisoning is usually accidental and is usually associated with low morbidity and mortality. It commonly occurs in a domestic environment and involves pesticides, disinfectants, alcoholic drinks and pharmaceutical products. In adults, poisoning is usually deliberate and has a higher morbidity and mortality rate (Meredith, 1993). Adult poisoning usually results from suicidal attempts due to social and economic stress and/or mental disorders.
Whereas poisoning in children is mainly accidental, deliberate poisoning by parents, guardians and siblings does occur (True, 2001).

Children less than 17 years of age account for most poisoning exposures but account for only about 10% of fatalities. A study conducted in hospitals in Kenya indicated that 40% of poisoning occurs in children aged below 14 years (Maitai, 1995). The common toxicants in this age category are kerosene (41%), pharmaceutical products (24%) and pesticides (15%).

Few hospitals in developing countries have the laboratory capacity to identify toxicants and in most cases, the diagnosis is made on circumstantial and clinical evidence. This includes details of drugs and other substances the patient had access to, a clinical picture of the present poisoning, and other substances the patient may have been exposed to (Leiken, 1998).

Acute pesticide poisoning is an important cause of worldwide morbidity and mortality. Globally, it has been estimated that there are three million cases of acute pesticide poisoning each year with some 220,000 deaths. Ninety-five percent of fatal pesticide poisonings occur in developing countries (Meredith, 1993). Pesticides account for close to one-third of all the cases of
poisoning received in Kenyan hospitals (Maitai, 1995). Organophosphates, which are widely available and commonly used in agriculture and in most households to control a wide range of pests, are the most common toxicants.

Amitraz is a synthetic \( \alpha_2 \) adrenergic agonist with wide spectrum insecticidal and acaricidal properties. It is sold and used worldwide as a veterinary and agricultural product to control a wide range of pests ranging from red spider mites on fruit crops to ticks and lice on livestock (Ellenhorn, 1997; Gosselin et al, 1984). It is registered in Kenya for use as an acaricide in tick control.

Commercial formulations of amitraz contain 12.5 – 20% of the drug in organic solvents, especially xylene. It is diluted in water before applying to plants and animals (Jones, 1990).

Amitraz causes poisoning in animals and humans when ingested, inhaled or after skin exposure. Cases of amitraz poisoning in humans has increased in recent years, probably due to widespread availability, increased use and it's relatively low cost. Poisoning has both been accidental and deliberate (Jorens, 1997).

When humans are exposed to amitraz, the symptoms and signs result both from xylene and amitraz. Poisoning presents with numerous signs ranging from central nervous system (CNS)
depression (drowsiness, coma and convulsion) to miosis, or rarely, mydriasis, respiratory depression, bradycardia, hypotension, hypertension, hypothermia or fever, hyperglycaemia, polyuria, vomiting, decreased gastrointestinal motility and intestinal distension (Ellenhorn, 1997). Xylene can cause acute toxic signs such as CNS depression, ataxia, impaired motor coordination, nystagmus, stupor, coma and episodes of neuro-irritability (Jones, 1990).

The signs and symptoms of amitraz poisoning are similar to those caused by opioids, organophosphates and centrally acting α2 adrenergic agonist drugs such as clonidine (Al-Qarawi, 1999). This may lead to diagnostic confusion and physicians should make a diagnosis based on information obtained from the patients or other informants, specific symptoms of poisoning and good toxicological screening.

There is no specific antidote for amitraz, although yohimbine and phentolamine may be useful (Upjohn, 1984). Treatment is primarily symptomatic and supportive. Effects of amitraz resemble those caused by pure alpha-2-adrenergic agonist drugs, and because of this mechanism, phentolamine or yohimbine have been suggested as therapies following a large ingestion (Harvey et al, 1998),
although data is lacking to confirm the effectiveness of these agents.

Amitraz is metabolised in the body to 3-methyl-4-aminobenzoic acid, which is conjugated and excreted in urine. Alkalinization of urinary pH is known to hasten excretion of acid toxicants and their metabolites. The hypothesis for the study is that since the primary metabolite of amitraz is an acid which is excreted in urine, it is possible to favour the excretion of the metabolite by alkalinising the urine and hence reduce the relative toxicity of amitraz.

Further, since yohimbine is an alpha-2-adrenergic antagonist, and therefore an antidote for amitraz which is an alpha-2-adrenergic agonist (Harvey, 1998), co-administration of amitraz and yohimbine would result in lowering the relative toxicity of amitraz.

The determination of the Median Lethal dose (LD₅₀) of a substance in an animal is an indicator of the relative toxicity of the substance. An increase in the LD₅₀ of amitraz in laboratory animals implies a reduction in the relative toxicity of the toxicant.

The increase in availability and use of a toxicant in a community is usually associated with an increase in the number of cases and prevalence of poisoning with that toxicant.
Cases of amitraz poisoning in Kenya have increased in recent years probably due to its relatively low cost and widespread availability.

The symptoms of amitraz poisoning can be easily confused with symptoms related to other widely available toxicants, and their management can be mixed up. The clinical signs and symptoms of amitraz poisoning are usually observed in the first 24 to 48 hours after ingestion of the toxicant. If poisoning is suspected, treatment is primarily supportive and symptomatic paying particular attention to monitoring respiratory and cardiac function.

The general objective of the study was to investigate the prevalence of amitraz poisoning in Kenyatta National Hospital and determine the effect of urinary alkalination on the relative toxicity of amitraz.
1.2 OBJECTIVES

The specific objectives of the study were

To study the prevalence amitraz poisoning in Kenyatta National Hospital

To determine the intraperitoneal median lethal dose (LD$_{50}$) of amitraz in rats.

To study the effect of yohimbine antagonism in the intraperitoneal median lethal dose of amitraz in rats.

To study the effect of urinary alkalinization on the intraperitoneal median lethal dose (LD$_{50}$) of amitraz in rats.
CHAPTER TWO:
REVIEW OF LITERATURE

2.1 DEFINITIONS AND TERMINOLOGY

Toxicology is the study of the adverse effects of chemicals on living organisms (Hardman, 2001). Clinical Toxicology deals with the assessment and medical management of persons exposed acutely or chronically to potentially harmful agents (Herfindal, 1996). It focuses on the effects of substances in patients caused by accidental poisonings or intentional overdoses of medications, drugs of abuse, household products or various other chemicals (Young, 1995). Forensic toxicology combines analytical chemistry and fundamental toxicology and is concerned with the medicolegal aspects of chemicals.

Poisoning is defined as a clinical toxicity secondary to accidental or intentional exposure. An overdose refers to an intentional exposure with the intent of causing self-injury or death.

Acute toxicity refers to effects that are directly related to ingestion of a toxic substance and observed within 24 hours. Chronic toxicity refers to the effects of ingesting small quantities of a substance
CHAPTER TWO:

REVIEW OF LITERATURE

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Acute toxicity refers to effects that are directly related to ingestion of a toxic substance and observed within 24 hours. Chronic toxicity refers to the effects of ingesting small quantities of a substance
over a long period of time leading to accumulation, toxic concentration and thus symptoms of poisoning (Moffat, 2004).

Toxicology of drugs and drug combinations refers to the study of unwanted effects caused by drugs, drug combinations and cosmetics. Toxicology of dependence producing substances is the study of toxicity that is caused by dependence producing substances, and includes smoking and its relation to lung cancer and cardiovascular disease and the misuse of psychoactive substances such as heroin, lysergic acid diethylamide (LSD) and alcohol.

Toxicology of foodstuffs and food additives is the study of poisoning caused by preservatives, colorants and flavourants. It also includes the study of malnutrition associated with hunger and obesity.

Industrial toxicology embraces all types of occupational poisoning in industries and aspects of health and safety. Skin and respiratory diseases are important in industrial toxicology.

Environmental toxicology focuses on the toxicological consequences of pollution in man and the changes in the biosphere and disturbances in biological equilibrium. Environmental toxicants are both chemical (where the environment is affected by chemicals) and physical (where the environment is affected by chemically inert...
waste products such as plastic packaging and radioactive waste) (Moffat, 2004).

The substances most frequently responsible for human poisoning are detergents, pharmaceutical products, cosmetics and personal care products, pesticides and other insecticides and petroleum distillates (Litovitz, 1999).

2.2 TOXICITY STUDIES

Three basic types of tests are performed on animals for the purpose of detecting toxic effects from chemicals. The tests differ primarily in their duration. Tests that require only single doses of chemicals are referred to as acute tests. More prolonged studies require that the chemical be given at least once daily for a period of about three months. Such studies are referred to as sub-acute or prolonged tests. Additional tests involving the administration of a chemical to animals daily for periods of one to two years are referred to as chronic toxicity tests (Loomis, 1970).

2.2.1 Acute studies

Acute studies are carried out on essentially all chemicals of any biological interest (Loomis, 1970). The chemical is given to an
animal on one, or at most, two occasions to determine the symptomatology consequent to administration and to determine the LD$_{50}$ of the compound. Initially the chemical is given to a single species and a similar test is later carried out on a second species of animal. The route of administration selected would be the intended route of administration to humans.

The sequence of effects following the administration of a compound are observed for a period of 6 to 8 hours for the appearance of symptoms and are again observed after 24 hours for evidence of persistent symptoms or lethal effect. However it is desirable to observe the animals for at least seven days in order to detect delayed effects which may not be apparent at the end of the 24 hour experiment.

2.2.2 Sub-acute studies

Sub-acute or prolonged studies are designed to simulate the conditions of intended use of the compound, the route of administration and the duration of use as nearly as possible. The animal species selected must allow the necessary blood and tissue samples to be feasibly and repeatedly obtained without harming the animal (Loomis, 1970). The species most commonly used for sub-
acute studies are the rat and the dog. All prolonged toxicity tests should utilize only healthy, suitably housed and cared-for animals. The duration for conducting sub-acute studies is variable, ranging from one week to up to three months. The duration is usually determined by the expected duration of use of the compound.

2.2.3 Long-term studies

Chronic or long-term toxicity tests on experimental animals are those that are carried on for periods in excess of 90 days, in which the chemical being tested is either administered in the diet of given at least once daily. The concentration of the chemical should be sufficiently small to allow it to be compatible with the biologic substance for infinite periods of time (Loomis, 1970).

A sufficiently large number of animals and adequate doses of compound must be used so that some of the animals will be affected and perhaps die as the experiment progresses. Prior to and during the course of the chronic toxicity test, clinical evaluation of the animals should be made daily, or at least weekly by persons knowledgeable in animal behaviour and symptomatology.

All animals in chronic toxicity studies are eventually subjected to complete pathological evaluation. Animals that die during the
experiment and those sacrificed at the end are examined by autopsy and tissue sections prepared from all types of tissue for histological examination. It is also common to obtain evidence regarding the reversible nature of chronically induced toxicity.

2.3 PESTICIDE POISONING

Pesticides are classified as insecticides, herbicides, rodenticides, molluscicides and acaricides, depending on their application. They come from a variety of chemical classes such as organophosphates, chlorinated hydrocarbons, carbamates and pyrethroids. Pesticides are applied in agriculture for crop protection and pest control and in human and animal hygiene.

Pesticides are classified by the World Health Organization (WHO) into five groups according to their relative acute oral toxicity (Tomlin, 2000).

Toxicity is determined on the basis of oral LD$_{50}$ for the rat and estimated lethal doses related to man (Table I). However, realistic human lethal doses of pesticides can be estimated only on the basis of well documented cases of actual poisoning.
Table I: WHO Toxicity Classification for Estimating Oral Acute Toxicity of Pesticides

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>Oral LD50 rats (mg/kg bwt)</th>
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<tr>
<td></td>
<td></td>
<td>Solids</td>
</tr>
<tr>
<td>Ia</td>
<td>Extremely hazardous</td>
<td>( \leq 5 )</td>
</tr>
<tr>
<td>Ib</td>
<td>Highly hazardous</td>
<td>5 - 50</td>
</tr>
<tr>
<td>II</td>
<td>Moderately hazardous</td>
<td>50 - 500</td>
</tr>
<tr>
<td>III</td>
<td>Slightly hazardous</td>
<td>( \geq 501 )</td>
</tr>
<tr>
<td>T5</td>
<td>Product unlikely to cause</td>
<td>hazard in normal use.</td>
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2.4 MANAGEMENT OF PESTICIDE POISONING IN HUMANS

Supportive therapy remains the cornerstone of management of acute poisoning. Patients who reach the hospital in time respond well to supportive therapy. It is designed to support respiration and cardiovascular function (True, 2001; Leikin, 1998).

Specific antidotes are available for metals (chelating agents), anticholinesterase inhibitors (atropine and pralidoxime), methanol (ethyl alcohol), paracetamol (acetyl cysteine) and opioids (Naloxone).

Techniques are also employed to increase the rate of elimination of poisons. These include adjusting of Urinary pH, haemodialysis and peritoneal dialysis.

Alkalinization of urine effectively increases the elimination of acid toxicants and their metabolites (Ellenhorn, 1997).

Biochemical tests that gauge the physiological status of the patient are also important for the immediate management of the condition, and together with history and clinical manifestation, provide the basis for acute management of poisoning.

Toxicological screening has limited usefulness in resource poor settings due to unavailability of facilities, untimely collection of
samples and untimely acquisition of results. Toxicological investigations are mainly used for their historical value (Leiken 2000).

2.5 AMITRAZ

Amitraz is a synthetic formamidine pesticide with a wide spectrum of activity that makes it appropriate for use in numerous conditions. It is used for the control of ticks, lice, mites and other insects in livestock (Ellenhorn, 1997). Amitraz has also been used to control red spider mites and other pests in fruit crops (Gosselin, 1984).

Amitraz is an alpha-2-adrenergic agonist and the observed clinical effects of poisoning are similar to effects caused by other centrally acting alpha-2-adrenergic agonists such as clonidine (Jones, 1990). When humans are exposed to poisoning, the reported effects are due both to xylene and amitraz (Ellenhorn, 1997). Reported effects resemble those caused by pure alpha-2-adrenergic agonist drugs, and include CNS depression (drowsiness, unconsciousness, coma), bradycardia, miosis, hyperglycaemia, hypotension, vomiting hypothermia, respiratory depression, seizures and transient increases in liver enzymes. In children, the onset of signs and symptoms following oral or dermal administration typically appear
within 30 to 150 minutes. One case of coma was reported 1 hour following ingestion of approximately 12.5 g of amitraz in solution. CNS depression in children appeared to improve within 6 to 24 hours. Most patients recover with supportive care. Death has been reported in several poisoning cases. Complete recovery usually occurs within 24 to 48 hours (Leiken, 1998).

2.5.1 FORMULA, PHYSICAL AND CHEMICAL PROPERTIES

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY (IUPAC) $N$-methylbis(2,4-xylyliminomethyl)amine

FORMULA

FORMULA: $C_{19}H_{23}N_3$

CHEMICAL ACTIVITY: Acaricides

               Insecticides
This compound exists as white or pale yellow monoclinic needles.

Molecular Weight is 293.45

2.5.2 FORMULATIONS OF AMITRAZ

Commercial preparations of amitraz generally contain 12.5 – 20% of the drug in organic solvents, usually 75% xylene (Jones, 1990). It is also available as an emulsifiable concentrate or as wetable powder.
Amitraz is also available in aromatic mixtures containing up to 2.5% of epichlorhydrin, or may be formulated in toluene (Grossman, 1993), xylene, propylene oxide, and a blend of alkyl benzene sulfonates and ethoxylated polyethers (Jones, 1990). Amitraz solutions are generally diluted 100 to 600-fold in water before applying to trees or livestock.

2.5.3 USES OF AMITRAZ

Amitraz is a formamidine pesticide with agricultural and veterinary uses as an acaricide and insecticide. It is used to control cattle ticks, mites, and other insects (lepidopterous species), and to treat generalized demodectic mange (Demodex canis) in dogs (Jones, 1990). It has been used on pear trees as a pesticide (Bonsall & Turnbull, 1983). Amitraz has also been used to control red spider mites and other pests in fruit crops (Gosselin, 1984).

2.5.4 TOXICOSIS OF AMITRAZ

The primary mechanism of toxicity is stimulation of pre- and postsynaptic alpha-2-adrenergic receptors. The amitraz metabolite BTS 27 271 (N-2,4-dimethyl phenyl-N-methyl formamidine) is a
partial agonist of alpha adrenergic receptors and an antagonist of norepinephrine receptors (Bonsall & Turnbull, 1983).

Amitraz interacts with high potency and specificity with alpha-2-adrenoreceptors in vitro and after in vivo administration in mouse models (Costa, 1988). Effects include CNS depression (drowsiness, unconsciousness and coma), bradycardia, miosis, hyperglycaemia, hypotension, vomiting hypothermia, respiratory depression, seizures and transient increases in liver enzymes. Death has been reported in several poisoning cases. Complete recovery usually occurs within 24 to 48 hours.

Most cases involved ingestion of amitraz/solvent mixtures; more mild toxicity has been reported after extensive but brief dermal exposure. Acute symptoms are partly attributable to the xylene or other solvents present in the formulation. Aspiration, CNS depression, myocardial sensitization, and eye/dermal irritation are some effects associated with xylene.

Respiratory depression has been reported in a minority of patients with amitraz poisoning. Mechanical ventilation may be required (Atabek, 2002; Yilmaz, 2003).
Hypothermia was reported in 4 of 8 paediatric cases of amitraz poisoning (Aydin, 1997). Hypotension has occurred in humans following ingestion of amitraz/petroleum distillate mixtures (Kennel, 1996). Bradycardia occurred in 6 of 8 paediatric cases of accidental amitraz ingestion (Aydin, 1997). It has also been reported following ingestion of amitraz/petroleum distillates (Kennel, 1996).

Miosis has been reported in humans following ingestion of amitraz/petroleum distillates (Garnier et al et al, 1998). Miosis was reported in 84% of 11 children who ingested a liquid concentrate formulation of amitraz (Yaramis, 2000). Miosis is an alpha-2-adrenergic agonist effect of amitraz. Bilateral mydriasis (responsive to light) has been reported in one human poisoning case (Jorens, 1997). Higher doses of amitraz (alpha-2-adrenergic agonist) result in mydriasis, while lower doses result in miosis (Garnier et al, 1998).

CNS depression, including drowsiness, unconsciousness and coma, is the most common presenting manifestation of amitraz poisoning (Atabek, 2002; Yaramis, 2000; Leung, 1999; Garnier et al, 1998). In several small case series of children with amitraz poisoning, drowsiness or lethargy were very common, developing in
81% to 100% of children (Kalyoncu, 2002; Yilmaz, 2003). CNS depression generally resolves within 24 hours, following supportive care (Atabek, 2002).

Vomiting after ingestion of amitraz or amitraz preparations which contain xylene may be expected (Bonsall & Turnbull, 1983; Aydin, 1997). Vomiting in most cases is due to the petroleum distillates mixed with amitraz in commercial preparations (Garnier et al, 1998). Poisonings may result in hypersalivation. (Bizovi et al, 1995) reported a 17% incidence of salivation in 24 amitraz toxicity cases.

Hyperglycaemia is a common effect of amitraz poisoning, due to alpha-2-adrenoceptor stimulation, which reduces insulin secretion (Garnier et al, 1998).
2.5.5 TOXICOKINETICS OF AMITRAZ

Most of the kinetics information is based on animal studies. Amitraz is readily absorbed following ingestion or dermal exposure (Grossman, 1993; Hsu, 1988). Blood levels peaked within 3 hours of ingestion in dogs. Onset of toxic effects ranged from 30 to 90 minutes after ingestions of undiluted amitraz solutions (12.5%) in children (Yaramis et al, 2000).

Highest tissue levels in animals are found in bile, liver, eye and intestine. Amitraz is rapidly metabolised in animals to N-2,4-dimethylphenyl-N-methylformamidine [BTS-27271] (Bonsall & Turnbull, 1983).

The principal final metabolite of amitraz in animals is 3-methyl-4-aminobenzoic acid, which is conjugated and excreted in the urine. Peak urine levels occurred 6 to 23 hours after administration to dogs. In a human adult case of estimated 250 mg amitraz ingestion, the elimination serum half-life was calculated to be 4 hours. No amitraz was detectable in the serum after 21 hours (Jorens et al, 1997).
2.5.6 MANAGEMENT OF AMITRAZ TOXICITY

There is no known antidote for amitraz (Upjohn, 1984). Treatment is primarily symptomatic and supportive. Effects of amitraz resemble those caused by pure alpha-2-adrenergic agonist drugs, and because of this mechanism, phentolamine or yohimbine have been suggested as therapies following a large ingestion (Harvey et al, 1998), although data is lacking to confirm the effectiveness of these agents.

Initial symptoms may be due to xylene or other solvents in the amitraz formulation. Primary solvent related effects may include respiratory distress due to aspiration and pneumonitis, apnoea, coma, hypotension, cardiac arrhythmia, hyperglycaemia and vomiting. Solvent formulations may vary in different countries.

Delayed cardiovascular (e.g., bradycardia, hypotension or hypertension), CNS depression, endocrine (e.g., hyperglycemia, altered insulin response) and other effects (e.g., hypothermia) may result from the amitraz component of the formulations.

Ipecac-induced emesis is not recommended because of the potential for CNS depression, seizures and cardiovascular
instability. Gastric lavage or nasogastric suction should be considered after large, recent ingestions. The potential for aspiration pneumonitis from xylene and the possibility of cardiac arrhythmias should be considered in the decision to lavage.

The decision to use activated charcoal should be based on the patient's status and on clinical judgement. Administration of activated charcoal 0.5 g/kg to rats, 30 minutes after oral dosing of amitraz or amitraz/xylene mixture, had no effect on survival or severity of symptoms (Turnbull, 1983). The manufacturer states that experimental studies have shown that systemic effects have not been ameliorated by absorption retardants (Upjohn, 1984).

Vital signs and ECG should be monitored regularly, as well as respiratory and central nervous system functions. The patient should be monitored for hypothermia and hyperthermia and warming provided for hypothermic patients. Cooling measures may be warranted with significant hyperthermia. Patients may require endotracheal intubation and mechanical ventilation because of respiratory depression, apnoea, mental status depression or coma during the first 24 hours following ingestion of amitraz (Aydin et al, 1997; Harvey et al, 1998). In cases of aspiration pneumonia, 100%
humidified supplemental oxygen is administered with assisted ventilation as required.

Atropine may be useful for treatment of haemodynamically unstable bradycardia (Aydin et al, 1997). Hypertension is generally transient and mild and may not require treatment. Aggressive therapy with hypotensive agents may result in profound and prolonged hypotension. If severe hypertension requires therapy short acting titratable agents are recommended.

Hyperglycaemia and glycosuria have been reported in humans exposed to amitraz. Animal studies have shown that amitraz inhibits insulin release in response to glucose challenge and may alter glucose metabolism (Smith et al, 1990).

2.6 DETERMINATION OF MEDIAN LETHAL DOSE

Several methods have been used in the determination of the median lethal dose (LD$_{50}$) in Laboratory animals. The term LD$_{50}$ refers to the least dosage that should be used to kill 50% of the
animals that received it. The methods used to determine LD$_{50}$ include the Arithmetic method of Reed and Muench (1938) and the Graphical method of Linchfield and Wilcoxon (1949). Whereas the graphical methods are more accurate and used in research, the arithmetic methods are fairly straightforward and are routinely used. In 1947, a method that involved the use of moving averages and interpolation to estimate the median effective dose was published (Thompson, 1947). The method would also be used to estimate the LD$_{50}$ if death is the critical response.

To reduce the time of calculation of the median lethal dose and its confidence interval to a minimum without the sacrifice of accuracy, a group of tables have been calculated according to formulae developed by Thompson et al (1952). The tables allow for the use of 2, 3, 4, 5, 6 or 10 animals per dose level, with 4 or more dosage level being tested per material, provided that the logarithms of successive dosage levels differ by a constant (Weil, 1952).

The requirements that must be followed to use the tables are:

1. A constant number of animals are dosed at each dose level ($n=$ number of animals dosed at each dose level).
2. The dosage levels are spaced so that they are in geometric progression. For example if the geometric factor (R) is 2.0, with dosage levels of 0.5, 1.0, 2.0 and 4.0 grams per kilogram body weight, then

\[ d = \log R = 0.30103 \]

where \( d \) is the logarithm of the ratio between dose levels

3. Animals should be dosed on at least \( K + 1 \) levels of dosages

i.e. 4 levels or more for \( K = 3 \).

When these requirements are followed, we seek to obtain from animals dosed at succeeding dosage levels a set of mortality data (r values) that match those provided in the tables for the given value of \( n \) animals and \( K \) dosage levels.

The general formula for the calculation of \( m \), the estimate of the LD\(_{50}\), is reduced to:

\[ \log m = \log D_a + d (f + 1) \quad \text{for } K = 3 \]

Where:
2. The dosage levels are spaced so that they are in geometric progression. For example if the geometric factor (R) is 2.0, with dosage levels of 0.5, 1.0, 2.0 and 4.0 grams per kilogram body weight, then

\[ d = \log R = 0.30103 \]

where \( d \) is the logarithm of the ratio between dose levels

3. Animals should be dosed on at least \( K + 1 \) levels of dosages

   i.e. 4 levels or more for \( K = 3 \).

When these requirements are followed, we seek to obtain from animals dosed at succeeding dosage levels a set of mortality data (\( r \) values) that match those provided in the tables for the given value of \( n \) animals and \( K \) dosage levels.

The general formula for the calculation of \( m \), the estimate of the \( LD_{50} \), is reduced to:

\[ \log m = \log D_a + d (f + 1) \quad \text{for } K = 3 \]

Where:
- $D_a$ is the dose of the lowest of the four dose levels used
- $d$ is the logarithm of the constant ratio between dosage levels
- the value of $f$ is obtained from the tables

To estimate the 95% confidence limits for the $LD_{50}$, we take the values bounded by

$$\text{antilog} \left[ \log m \pm 2 \delta_{\log m} \right]$$

Where $\delta_{\log m} = d \cdot \delta_f$

The use of these tables allows the simple and rapid estimation of $LD_{50}$ and a corresponding confidence interval.
CHAPTER THREE:
MATERIALS AND METHODS

4.5 DEMOGRAPHIC SURVEY OF POISONING AT KENYATTA NATIONAL HOSPITAL WITH EMPHASIS TO AMITRAZ

3.1.1 INTRODUCTION

Kenyatta National Hospital is a regional referral, research and teaching hospital located in the Upper hill region in Nairobi. It receives patients on referral from other hospitals and institutions within and outside Kenya for specialised health care and acute cases from within Nairobi. The hospital also provides facilities for medical education for the University of Nairobi and training of nurses and other health and allied professions. It provides facilities for medical research either directly or in collaboration with other institutions. The hospital is also involved in national health planning. Kenyatta National Hospital receives an average of 570,000 outpatients and 78,000 inpatients every year.
3.1.2 ETHICAL APPROVAL

Approval was sought from the Kenyatta National Hospital Ethics and Research Committee (ERC) for the study. A research proposal was presented to the committee detailing procedures to be used in data retrieval and collection and commitment to ensure patient confidentiality. Approval was granted and the study authorised to be conducted for a period of one year (Appendix I).

3.1.3 DATA RETRIEVAL

The Medical records department at Kenyatta National Hospital codes and classifies all medical and surgical conditions of patients according to the World Health Organization International Coding of Diseases (ICD) system. The ICD-10 classification code for Pesticide poisoning was identified and all the patient files for the period under study retrieved with assistance from staff at the Medical Records Department.

3.1.4 DATA COLLECTION

A data collection form (Appendix II) was designed to summarize information obtained from patient's records. The data obtained included:
- Case number (Patient's hospital reference number)
- Age (in years)
- Sex
- Place of poisoning (Rural or urban)
- Location of Poisoning (Home, work or social setting)
- Socio-economic level (Low, Middle or High)
- Identity of toxicant
- Type of Exposure (Accidental, Intentional or undetermined)
- Route of poisoning (Oral, Dermal or other)
- Major presenting Clinical signs
- Length of stay in Hospital (Days)
- Outcome (Cured or died)
- Remarks on management

The information required was retrieved from the patient files and summarized in the data collection forms for the period of the study. The prevalence of amitraz poisoning in Kenyatta National Hospital was determined by reviewing Medical records available for patients admitted with poisoning for a 6 year period from January 2000 to December 2006. The results were compared with poisoning by other toxicants during the same period of time and tabulated. A comparative analysis was performed.
3.2 TOXICOLOGICAL STUDY OF AMITRAZ POISONING IN RATS

3.2.1 MATERIALS

3.2.1.1 RATS

200 weaned white albino rats of both sexes were obtained from the Veterinary research laboratories in Kabete and transported to the University of Nairobi, Department of Pharmacology and Toxicology animal house. The animals were separated into male and female to avoid breeding and housed in cages with wood shaving bedding. They were fed on ground pellets (Unga Limited, Nairobi) and water ad libitum for a period of 7 to 10 days to allow for acclimatization to their surroundings.

3.2.1.2 CHEMICALS

Amitraz was obtained from Coopers Kenya Ltd as Triatix®. The formulation is available as an Emulsifiable Concentrate (EC) containing 12.5% w/v amitraz.

Yohimbine was obtained as a sterile parenteral preparation containing 6.25 mg/ml of the active ingredient.

Sodium bicarbonate was obtained as a sterile parenteral preparation of 7.5% w/v NaHCO₃ from M/s Lab Renaudin, France.
3.2.2 PRELIMINARY STUDIES

DOSE RANGE FINDING TESTS

A total of 30 animals were used in the preliminary studies to estimate the intraperitoneal toxic dose range for amitraz.

Six groups of 5 rats were randomly selected from both sexes and placed in separate labelled cages corresponding to different dose groups. The animals were fasted overnight. Each animal was weighed and given intraperitoneal amitraz using a 1 ml syringe and 26 gauge needle at doses of 12.5, 32, 60, 90, 133 and 200 mg/kg body weight according to its dose group. The dose levels were determined using a geometric factor of 1.5 starting from the highest dose. Acute toxicity was observed after 24 hours and the information used as a guideline for determination of LD$_{50}$.

RESULTS OF PRELIMINARY STUDIES

The numbers of rats that survived or died in 24 hours were recorded for each of the dose groups (Table 6).
Table 2: Preliminary determination of IP LD50 of Amitraz in Rats: Death/survival at 24 hours.

<table>
<thead>
<tr>
<th>Dose group</th>
<th>No</th>
<th>Sex</th>
<th>Weight (g)</th>
<th>Dose (mg/kg)</th>
<th>Volume (ml)</th>
<th>Outcome (24 hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>1</td>
<td>F</td>
<td>190</td>
<td>12.5</td>
<td>0.019</td>
<td>Alive</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>M</td>
<td>158</td>
<td>12.5</td>
<td>0.016</td>
<td>Alive</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>M</td>
<td>153</td>
<td>12.5</td>
<td>0.016</td>
<td>Alive</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>M</td>
<td>172</td>
<td>12.5</td>
<td>0.018</td>
<td>Alive</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>M</td>
<td>188</td>
<td>12.5</td>
<td>0.019</td>
<td>Alive</td>
</tr>
<tr>
<td>32</td>
<td>1</td>
<td>M</td>
<td>293</td>
<td>32</td>
<td>0.075</td>
<td>Alive</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>M</td>
<td>206</td>
<td>32</td>
<td>0.053</td>
<td>Dead</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>M</td>
<td>290</td>
<td>32</td>
<td>0.074</td>
<td>Alive</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>M</td>
<td>320</td>
<td>32</td>
<td>0.082</td>
<td>Alive</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>M</td>
<td>162</td>
<td>32</td>
<td>0.041</td>
<td>Alive</td>
</tr>
<tr>
<td>60</td>
<td>1</td>
<td>M</td>
<td>280</td>
<td>60</td>
<td>0.13</td>
<td>Alive</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>F</td>
<td>214</td>
<td>60</td>
<td>0.10</td>
<td>Alive</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>M</td>
<td>346</td>
<td>60</td>
<td>0.17</td>
<td>Alive</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>M</td>
<td>335</td>
<td>60</td>
<td>0.16</td>
<td>Alive</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>F</td>
<td>289</td>
<td>60</td>
<td>0.14</td>
<td>Dead</td>
</tr>
<tr>
<td>Dose No</td>
<td>Sex</td>
<td>Weight (g)</td>
<td>Dose (mg/kg)</td>
<td>Volume (ml)</td>
<td>Outcome</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-----</td>
<td>------------</td>
<td>--------------</td>
<td>-------------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>1</td>
<td>M 287</td>
<td>90</td>
<td>0.21</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>143</td>
<td>90</td>
<td>0.10</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>223</td>
<td>90</td>
<td>0.16</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>303</td>
<td>90</td>
<td>0.22</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>189</td>
<td>90</td>
<td>0.14</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>133</td>
<td>1</td>
<td>F 278</td>
<td>133</td>
<td>0.303</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>237</td>
<td>133</td>
<td>0.26</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>194</td>
<td>133</td>
<td>0.21</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>346</td>
<td>133</td>
<td>0.38</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>220</td>
<td>133</td>
<td>0.24</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>1</td>
<td>M 260</td>
<td>200</td>
<td>0.42</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>267</td>
<td>200</td>
<td>0.43</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>266</td>
<td>200</td>
<td>0.43</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>192</td>
<td>200</td>
<td>0.31</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>321</td>
<td>200</td>
<td>0.51</td>
<td>Dead</td>
<td></td>
</tr>
</tbody>
</table>

**INTERPRETATION OF PRELIMINARY STUDIES**

The preliminary results indicate that the intraperitoneal LD$_{50}$ is within the range of 12.5 to 200mg/kg body weight.
3.2.3 EXPERIMENT 1: DETERMINATION OF INTRAPERITONEAL MEDIAN LETHAL DOSE FOR AMITRAZ IN RATS

40 rats were divided into 4 groups of 10 animals each corresponding to 4 dose levels. The dose levels were determined using a geometric factor of 1.5 from the lowest dose to the highest and were guided by the results obtained from the preliminary dose range finding experiment. The animals were weighed and the amitraz was administered intraperitoneally using a 1ml syringe and a 26 gauge needle.

Calculations

The volume of amitraz administered was calculated as follows:

\[
\text{Volume (ml)} = \frac{\text{Body weight (kg) \times Dosage (mg/kg body weight)}}{\text{Concentration (mg/ml)}}
\]

The concentration of amitraz used was 12.5% w/v.

Hence 100ml of the formulation contains 12.5g (or 12500 mg) of amitraz.
1 ml of solution will therefore contain \((12500 \times \frac{1}{100})\) or 125 mg of amitraz.

For example, for an animal weighing 250g receiving amitraz at a dosage rate of 200mg/kg body weight:

Volume of amitraz (ml) = \(0.250\text{kg} \times \frac{200\text{mg}}{\text{kg}} = 0.40\text{ ml}\)

The number of rats that survived or died within 24 hours for each of the dosages was recorded (Table 7) and used to calculate the \(LD_{50}\) using tables for convenient calculation of median lethal dose by Weil.

3.2.5 EXPERIMENT 2: A STUDY OF THE EFFECT OF YOHIMBINE ANTAGONISM ON THE INTRAPERITONEAL MEDIAN LETHAL DOSE OF AMITRAZ IN RATS.

40 rats were divided into 4 groups of 10 animals each corresponding to 4 dose levels. The dose levels were determined
using a geometric factor of 1.5 from the lowest dose to the highest and were guided by the results obtained from the preliminary dose range finding experiment. The animals were weighed and the amitraz was administered intraperitoneally using a 1ml syringe and a 26-gauge needle followed by yohimbine intraperitoneally at a therapeutic dose of 0.5mg/kg body weight.

**Calculations**

The volume of amitraz administered was calculated as in the previous experiment.

Yohimbine was available as a formulation containing 6.25mg/ml. The therapeutic dosage for yohimbine is 0.125 to 0.5mg/kg.

Using the higher therapeutic dose,

\[
\text{Volume of yohimbine (ml)} = \text{body wt (kg)} \times \text{dosage (mg/kg)}
\]

\[
6.25 \text{ (mg/ml)}
\]

The number of rats that survived or died within 24 hours for each of the dosages was recorded (Table 8) and used to calculate the \(LD_{50}\) using tables for convenient calculation of median lethal dose by Weil (1952).
3.2.5 EXPERIMENT 3: A STUDY OF THE EFFECT OF URINARY ALKALINATION ON THE INTRAPERITONEAL MEDIAN LETHAL DOSE OF AMITRAZ IN RATS.

40 rats were divided into 4 groups of 10 animals each corresponding to 4 dose levels. The dose levels were determined using a geometric factor of 1.5 from the lowest dose to the highest and were guided by the results obtained from the preliminary dose range finding experiment. The animals were weighed and the amitraz was administered intraperitoneally using a 1ml syringe and a 26-gauge needle followed by Sodium bicarbonate intraperitoneally at a therapeutic dose of 50mg/kg body weight.

Calculations

The volume of amitraz administered was calculated as in the previous experiment.

Sodium bicarbonate was available as a sterile solution containing 7.5% w/v.

Hence 100ml of the formulation contains 7.5g (or 7500 mg) of NaHCO₃.
1 ml of solution will therefore contain \((7500 \times 1/100)\) or 75 mg of NaHCO₃.

The therapeutic dosage for urinary alkalization using NaHCO₃ is 50mg/kg.

Using the higher therapeutic dose,

\[
\text{Volume of NaHCO}_3 (\text{ml}) = \text{body wt (kg)} \times 50 \text{ (mg/kg)} \times \frac{75 \text{ (mg/ml)}}{} 
\]

The number of rats that survived or died within 24 hours for each of the dosages was recorded (Table 8) and used to calculate the LD₅₀ using tables for convenient calculation of Median lethal dose by Weil (1952).
CHAPTER FOUR:

RESULTS

4.1 DEMOGRAPHIC SURVEY OF POISONING AT KENYATTA NATIONAL HOSPITAL

A total of 1004 patients' files were studied. Files that were not available at the Medical records at the time of the study were excluded (Table 3). Of this, 60.7% (609/1004) of the cases were male while 39.3% (395/1004) were female (Table 5).

A total of 78.3% (577/737) of the poisoning cases received occurred in an urban setting, probably due to the urban location of the hospital. About 93.4% (688 of 737) of the cases of poisoning reviewed occurs in a home setting compared to 2.4% that occur at work and 4.2% in social settings.

The average length of stay in hospital is 5.6 days (range: one to 56 days). Of the patients studied, 90.1% (905/1004) of the patients admitted recovered compared to 9.9% (99/1004) who died.
Table 3: Pesticide Poisoning at Kenyatta National Hospital according to year and toxicant

<table>
<thead>
<tr>
<th>Identity of Toxicant</th>
<th>Year</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organophosphate</td>
<td></td>
<td>43</td>
<td>31</td>
<td>56</td>
<td>55</td>
<td>45</td>
<td>52</td>
<td>282</td>
</tr>
<tr>
<td>Rodenticides</td>
<td></td>
<td>16</td>
<td>38</td>
<td>38</td>
<td>80</td>
<td>71</td>
<td>47</td>
<td>290</td>
</tr>
<tr>
<td>Amitraz</td>
<td></td>
<td>10</td>
<td>7</td>
<td>10</td>
<td>10</td>
<td>14</td>
<td>18</td>
<td>69</td>
</tr>
<tr>
<td>Other insecticide</td>
<td></td>
<td>6</td>
<td>7</td>
<td>11</td>
<td>11</td>
<td>14</td>
<td>8</td>
<td>57</td>
</tr>
<tr>
<td>Not identified</td>
<td></td>
<td>27</td>
<td>39</td>
<td>14</td>
<td>27</td>
<td>18</td>
<td>7</td>
<td>132</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>102</td>
<td>122</td>
<td>129</td>
<td>183</td>
<td>162</td>
<td>132</td>
<td>830</td>
</tr>
</tbody>
</table>

1 Excludes files that were unavailable at the Medical records Department during the course of the study
Figure 1: Pie chart showing type of pesticide poisoning encountered at Kenyatta National Hospital
Figure 2: Bar graph showing type of pesticide poisoning encountered in Kenyatta National Hospital from 2000 to 2005.
Table 4: Pesticide Poisoning at Kenyatta National Hospital from 2000 to 2005 according to age and toxicant\textsuperscript{2}

<table>
<thead>
<tr>
<th>Identity of Toxicant</th>
<th>Age (years)\textsuperscript{3}</th>
<th>0-5</th>
<th>6-12</th>
<th>13-20</th>
<th>21-30</th>
<th>31-40</th>
<th>41-50</th>
<th>51+</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organophosphate</td>
<td></td>
<td>18</td>
<td>1</td>
<td>48</td>
<td>143</td>
<td>41</td>
<td>13</td>
<td>5</td>
<td>269</td>
</tr>
<tr>
<td>Rodenticides</td>
<td></td>
<td>8</td>
<td>2</td>
<td>92</td>
<td>155</td>
<td>21</td>
<td>4</td>
<td>3</td>
<td>285</td>
</tr>
<tr>
<td>Amitraz</td>
<td></td>
<td>14</td>
<td>3</td>
<td>10</td>
<td>29</td>
<td>9</td>
<td>3</td>
<td>0</td>
<td>68</td>
</tr>
<tr>
<td>Other insecticide</td>
<td></td>
<td>8</td>
<td>1</td>
<td>11</td>
<td>27</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>58</td>
</tr>
<tr>
<td>Not identified</td>
<td></td>
<td>24</td>
<td>3</td>
<td>22</td>
<td>46</td>
<td>14</td>
<td>6</td>
<td>5</td>
<td>120</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>72</td>
<td>10</td>
<td>183</td>
<td>400</td>
<td>93</td>
<td>28</td>
<td>14</td>
<td>800</td>
</tr>
</tbody>
</table>

\textsuperscript{2} Excludes files that were unavailable at the Medical records Department during the course of the study.

\textsuperscript{3} Excludes patients' files where the age is simply indicated as "Adult".
Figure 3: Line graph indicating distribution of Pesticide Poisoning from 2000 to 2005 at Kenyatta National Hospital by age
Table 5: Pesticide poisoning at Kenyatta National Hospital from 2000 to 2005 according to age and sex.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0-5</td>
<td>6-12</td>
</tr>
<tr>
<td>2000</td>
<td>Male</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>2001</td>
<td>Male</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>2002</td>
<td>Male</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>2003</td>
<td>Male</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2004</td>
<td>Male</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>2005</td>
<td>Male</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>89</td>
<td>15</td>
</tr>
</tbody>
</table>

*Excludes patients' files where the age is simply indicated as "Adult".*
Figure 4: Bar Graph comparing distribution of Pesticide Poisoning at Kenyatta National Hospital by sex and age
Table 6: Pesticide Poisoning in Kenyatta National Hospital according to age and type of exposure

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Intentional</th>
<th>Accidental</th>
<th>Undetermined</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Suicide</td>
<td>Homicide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – 5</td>
<td>0</td>
<td>13</td>
<td>49</td>
<td>7</td>
</tr>
<tr>
<td>6 – 12</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>12 – 20</td>
<td>147</td>
<td>1</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>21 – 30</td>
<td>338</td>
<td>1</td>
<td>19</td>
<td>30</td>
</tr>
<tr>
<td>31 – 40</td>
<td>73</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>41 – 50</td>
<td>17</td>
<td>0</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>51 +</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>591</td>
<td>16</td>
<td>90</td>
<td>68</td>
</tr>
</tbody>
</table>

1 Excludes files that were unavailable at the Medical records Department during the course of the study

2 Excludes patients’ files where the age is simply indicated as “Adult”.
Figure 5: Bar graph indicating distribution of Poisoning at Kenyatta National Hospital by age and type of exposure.
4.5 EXPERIMENT 1: INTRAPERITONEAL LD$_{50}$ OF AMITRAZ IN RATS

Table 7: Death and survival at 24 hours after IP administration of Amitraz

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals (n)</th>
<th>Dose (mg/kg)</th>
<th>No. Dead (a)</th>
<th>No. Survived (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>170</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>113</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>75</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>50</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

CALCULATIONS

From Table 7 above, the intraperitoneal LD$_{50}$ for amitraz in rats is calculated as follows:
The Weil's tables for calculating \( LD_{50} \) uses the formula:

\[
\log m = \log LD_{50} = \log D_a + d (f + 1)
\]

where

\[
\log D_a = \text{logarithm of lowest of the four dosage levels used}
\]

\[
d = \text{logarithm of the constant ratio between dosage levels}
\]

\[
n = \text{number of animals per dosage levels} = 10,
\]

\[
K = 3 \text{ for } 4 (K+1) \text{ dosage levels.}
\]

\[
m = \text{median lethal dose}
\]

Referring to the \([2, 5, 8, 10]\) r-value in the \(n=10\) and \(K=3\) section of the tables,

\[
f = 0.0000 \text{ and } \delta_f = 0.31458
\]

Hence

\[
\log LD_{50} = \log D_a + d (f + 1) = \log 50 + \log 1.5 (0.000 + 1)
\]

\[
\log LD_{50} = 1.699 + 0.1761 = 1.8751
\]
LD_{50} = \text{antilog (1.8751)} = 75.01 \text{mg/kg}

The intraperitoneal LD_{50} for amitraz in rats 75.01 mg/kg body weight.

The 95% confidence limit for the LD_{50} are bounded by

\text{antilog [log m} \pm 2 \delta_{\log m}] \text{]

where \delta_{\log m} = d \times \delta_{f}

= \text{antilog [log 75.01} \pm 2 \times 0.1761 \times 0.31458]\text{]

= \text{antilog [1.875 - 0.1109] to antilog [1.875 + 0.1109]}

= \text{antilog (1.7641) to antilog (1.9859)}

The 95% confidence limits are bounded by 58.1 mg/kg body weight and 96.8 mg/kg body weight.

The intraperitoneal LD_{50} for amitraz in rats and its 95% confidence limit is 75.01 (58.1 to 96.8)mg/kg.
4.5 EXPERIMENT 2: THE EFFECT OF YOHIMBINE ANTAGONISM ON INTRAPERITONEAL LD50 OF AMITRAZ IN RATS

Table 8: Death and survival at 24 hours after IP administration of amitraz and yohimbine 0.5mg/kg

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Dose of amitraz (mg/kg)</th>
<th>No. Dead</th>
<th>No. Survived</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>175</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>113</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>75</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>50</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>
CALCULATIONS

From Table 8 above, the intraperitoneal LD$_{50}$ for amitraz with yohimbine antagonism in rats is calculated as follows:

The Weil's tables for calculating LD$_{50}$ uses the formula:

\[ \log m = \log LD_{50} = \log D_a + d (f + 1) \]

where

\[ \log D_a = \text{logarithm of lowest of the four dosage levels used} \]

\[ d = \text{logarithm of the constant ratio between dosage levels} \]

\[ n = \text{number of animals per dosage levels} = 10, \]

\[ K = 3 \text{ for } 4 (K+1) \text{ dosage levels.} \]

\[ m = \text{median lethal dose} \]

Referring to the [0, 6, 8, 10] r-value in the n=10 and K=3 section of the tables,

\[ f = 0.1 \text{ and } \delta_f = 0.21082 \]

\[ \log LD_{50} = \log D_a + d (f + 1) = \log 50 + \log 1.5 \times (0.1 + 1) \]
\[ \log \text{LD}_{50} = 1.699 + 0.1761 \times 1.1 = 1.89271 \]

\[ \text{LD}_{50} = \text{antilog (1.89271)} = 78.11 \text{mg/kg} \]

The intraperitoneal LD$_{50}$ for amitraz in rats following yohimbine antagonism is 78.11 mg/kg body weight.

The 95% confidence limit for the LD$_{50}$ are bounded by

\[ \text{antilog} [\log m \pm 2 \delta_{\log m}] \]

where \( \delta_{\log m} = d \times \delta_f \)

\[ = \text{antilog} [\log 78.11 \pm 2 \times 0.1761 \times 0.21082] \]

\[ = \text{antilog} [1.89271 - 0.0742] \text{ to } \text{antilog} [1.89271 + 0.0742] \]

\[ = \text{antilog} (1.8185) \text{ to } \text{antilog} (1.9669) \]

The 95% confidence limits are bounded by 65.84 mg/kg body weight and 92.66 mg/kg body weight.

The intraperitoneal LD$_{50}$ for amitraz following yohimbine antagonism in rats and its 95% confidence limit is 78.11 (65.84 to 92.66) mg/kg.
4.5 A STUDY OF THE EFFECT OF URINARY ALKALINATION ON INTRAPERITONEAL LD50 OF AMITRAZ IN RATS

Table 9: Death and survival at 24 hours after IP administration of Amitraz and Sodium Bicarbonate 50mg/kg:

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Dose of Amitraz (mg/kg)</th>
<th>No. Dead</th>
<th>No. Survived</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>170</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>113</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>75</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>50</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>
CALCULATIONS

From Table 9 above, the intraperitoneal LD$_{50}$ for amitraz following urinary alkalination in rats is calculated as follows:

The Weil's tables for calculating LD$_{50}$ uses the formula:

$$\log m = \log LD_{50} = \log D_a + d (f + 1)$$

where

$$\log D_a = \text{logarithm of lowest of the four dosage levels used}$$

$$d = \text{logarithm of the constant ratio between dosage levels}$$

$$n = \text{number of animals per dosage levels} = 10,$$

$$K = 3 \text{ for 4 (K+1) dosage levels.}$$

$$m = \text{median lethal dose}$$

Referring to the [2, 3, 6, 10] r-value in the n=10 and K=3 section of the tables,

$$f = 0.5 \text{ and } \delta_f = 0.2856$$

$$\log LD_{50} = \log D_a + d (f + 1) = \log 50 + \log 1.5 (0.5 + 1)$$
\[
\log \text{LD}_{50} = 1.699 + 0.1761 \times 1.5 = 1.9631
\]

\[
\text{LD}_{50} = \text{antilog} (1.9631) = 91.85 \text{ mg/kg body weight.}
\]

The intraperitoneal LD\(_{50}\) for amitraz in rats following urinary alkalination using Sodium bicarbonate is 91.85 mg/kg body weight.

The 95% confidence limit for the LD\(_{50}\) are bounded by

\[
\text{antilog} \left[ \log m \pm 2 \delta_{\log m} \right]
\]

where \(\delta_{\log m} = d \times \delta_f\)

\[
= \text{antilog} \left[ \log 91.85 \pm 2 \times 0.1761 \times 0.28565 \right]
\]

\[
= \text{antilog} [1.9631 - 0.1006] \text{ to antilog } [1.9631 + 0.1006]
\]

\[
= \text{antilog} (1.8625) \text{ to antilog } (2.0637)
\]

The 95% confidence limits are bounded by 72.86 mg/kg body weight and 115.8 mg/kg body weight.

The intraperitoneal LD\(_{50}\) for Amitraz in rats following urinary alkalination with Sodium bicarbonate and its 95% confidence limit is 91.85 (72.86 to 115.8) mg/kg.
4.5 CALCULATION OF SIGNIFICANCE

The experiments conducted indicate the following:

1. The intraperitoneal LD$_{50}$ for amitraz in rats and its 95% confidence limit is 75.01 (58.1 to 96.8)mg/kg.

2. The intraperitoneal LD$_{50}$ for amitraz in rats following yohimbine antagonism and its 95% confidence limit is 78.11 (65.84 to 92.66) mg/kg.

3. The intraperitoneal LD$_{50}$ for Amitraz in rats following urinary alkalination with Sodium bicarbonate and its 95% confidence limit is 91.85 (72.86 to 115.8) mg/kg.
CALCULATIONS:

1. Question to be answered: is there any difference between the intraperitoneal median lethal dose ($LD_{50}$) of Amitraz and the intraperitoneal median lethal dose of Amitraz following yohimbine antagonism?

Data:

<table>
<thead>
<tr>
<th></th>
<th>Amitraz</th>
<th>Amitraz + yohimbine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>$n_1 = 10$</td>
<td>$n_2 = 10$</td>
</tr>
<tr>
<td>$LD_{50}$ (calculated)</td>
<td>$X_1 = 75.01$</td>
<td>$X_2 = 78.11$</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>$\delta_1 = 0.31458$</td>
<td>$\delta_2 = 0.21085$</td>
</tr>
</tbody>
</table>

Hypothesis:

$H_0$: there is no difference in the intraperitoneal $LD_{50}$ of amitraz in rats following yohimbine antagonism.

$H_1$: there is a difference in the intraperitoneal $LD_{50}$ of amitraz in rats following yohimbine antagonism.
The Standard error of the difference in means (SE) is given by the formula:

\[
SE = s \sqrt{\left( \frac{1}{n_1} + \frac{1}{n_2} \right)}
\]

where

\[
s = \sqrt{\frac{(n_1 - 1)\sigma_1^2 + (n_2 - 1)\sigma_2^2}{n_1 + n_2 - 2}}
\]

In this problem

\[
s = \sqrt{\frac{(10 - 1)0.31458^2 + (10 - 1)0.21082^2}{10 + 10 - 2}}
\]

\[
= \sqrt{\frac{0.8906 + 0.4}{18}} = 0.2677
\]

Hence

\[
SE = 0.2677 \sqrt{\frac{1}{10} + \frac{1}{10}} = 0.1197
\]

The critical ratio, \( t \), is given by the equation:

\[
t = \frac{|X_1 - X_2|}{SE} = \frac{|75.01 - 78.1|}{0.1197} = 3.11
\]

Hence

\[t = 25.9\]
The critical ratio denoted by $t$ follows a t-distribution with $(n_1+n_2-2)$ degrees of freedom. The t-distribution for 18 degrees of freedom gives the 5% level as 2.101 and the 1% level as 2.878.

Our observed value of 25.9 is greater than both the 1% level and the 5% level, so we reject the null ($H_0$) hypothesis.

Hence, there is a significant difference between the intraperitoneal $LD_{50}$ of amitraz and the intraperitoneal $LD_{50}$ of amitraz following yohimbine antagonism ($p < 0.01$).

2. **Question to be answered**: is there any difference between the intraperitoneal median lethal dose ($LD_{50}$) of amitraz and the intraperitoneal median lethal dose of amitraz following urinary alkalination with Sodium bicarbonate?

Data:

<table>
<thead>
<tr>
<th></th>
<th>Amitraz</th>
<th>Amitraz + NaHCO$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample size</strong></td>
<td>$n_1 = 10$</td>
<td>$n_2 = 10$</td>
</tr>
<tr>
<td><strong>$LD_{50}$ (calculated)</strong></td>
<td>$X_1 = 75.01$</td>
<td>$X_2 = 91.85$</td>
</tr>
<tr>
<td><strong>Standard deviation</strong></td>
<td>$\delta_1 = 0.31458$</td>
<td>$\delta_2 = 0.28565$</td>
</tr>
</tbody>
</table>
Hypothesis:

$H_0$: there is no difference in the intraperitoneal LD$_{50}$ of amitraz in rats following urinary alkalination with NaHCO$_3$.

$H_1$: there is a difference in the intraperitoneal LD$_{50}$ of amitraz in rats following urinary alkalination with NaHCO$_3$.

The Standard error of the difference in means (SE) is given by the formula:

$$SE = \sqrt{s^2 \left( \frac{1}{n_1} + \frac{1}{n_2} \right)}$$

where $s = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}$

In this problem

$$s = \sqrt{(10 - 1)0.31458^2 + (10 - 1)0.28565^2}$$

$$= \sqrt{0.8906 + 0.734} = 0.3$$

Hence

$$SE = 0.3 \cdot \sqrt{\frac{1}{10} + \frac{1}{10}} = 0.1342$$

The critical ratio, $t$, is given by the equation:
\[ t = \frac{|X_1 - X_2|}{SE} = \frac{75.01 - 91.85}{0.1342} = \frac{16.84}{0.1342} \]

Hence

\[ t = 125.4 \]

The critical ratio denoted by t follows a t-distribution with \((n_1+n_2-2)\) degrees of freedom. The t-distribution for 18 degrees of freedom gives the 5% level as 2.101 and the 1% level as 2.878.

Our observed value of 125.4 is greater than both the 1% level and the 5% level, so we reject the null (H0) hypothesis.

Hence, there is a significant difference between the intraperitoneal LD50 of amitraz and the intraperitoneal LD50 of amitraz following urinary alkalination with NaHCO3 (p < 0.01).
CHAPTER 5
DISCUSSION AND CONCLUSION

5.1 DISCUSSION

There is a large variety of chemical compounds that exhibit pesticide properties. There are more than 1000 pesticides available and widely used in the world today; they include a wide range of chemical classes such as chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids (Moffat, 2004). In addition, several hundreds of compounds are no longer in use. Pesticides are applied in agriculture for crop protection and pest control and in human and animal hygiene.

Acute pesticide poisoning is an important cause of worldwide morbidity and mortality. It is estimated that there are three million cases of acute pesticide poisoning each year with some 220,000 deaths (Meredith, 1993). Most of the fatalities occur in developing countries.

The causes of poisoning are either accidental or deliberate. In the cases reviewed in the study, 79% of the cases are deliberate compared to 11% that are accidental (Table 6).
Poisoning usually takes place in a domestic environment (Moffat, 2004); of the cases reviewed, 93.4% occurred at home compared to 2.4% occurring at work and 4.2% occurring at social settings. While poisoning occurs at all ages, serious cases of pesticide poisoning are more likely to occur in adults than in children (Meredith, 1993). This agrees with the data obtained where poisoning occurs at all ages and peaks for all types of toxicants in the 21 to 30 age category (Figure 6). This can be linked to the fact deliberate poisoning usually results from suicidal attempts due to social and economic stress and/or mental disorders that are common in this age category (True et al, 2001).
In the age categories reviewed in the study, Poisoning was more frequent among men than women, with exception of the 13 to 20 age category. Here, poisoning was more among women as a result of deliberate suicidal attempts due to teenage pregnancy (Table 4 and Figure 3).
Poisoning in children is mainly accidental, but deliberate (homicidal) poisoning by parents, guardians and siblings, though rare, does occur (True et al, 2001). In the cases reviewed, 49 out of 69 (71%) of poisoning in the 0 to 5 age category were accidental and 13 (19%) homicidal (Table 6 and Figure 5).
Since only a few hospitals have laboratories with toxicology facilities, a vast majority of poisoning diagnosis is made by circumstantial and clinical evidence (Ellenhorn 1997, Moffat 2004). Biochemical studies that gauge the physiological status of the patient together with the history and clinical manifestation usually provide the basis of acute management of poisoning (Leiken, 1998).

In the cases surveyed, laboratory support was used to help define the physiological status of the patient; where toxicological evaluation was done, it was for historical value and medico-legal purposes. Supportive therapy remains the cornerstone of management of acute poisoning (Moffat, 2004). It is designed to support respiratory and cardiovascular function (True et al, 2001; Leiken, 1998). Techniques that increase the rate of elimination of poisons, such as diuresis, adjusting of urinary pH and dialysis have also been used in management of poisoning (Moffat, 2004). Specific antidotes are available for metals, anticholinesterase inhibitors, paracetamol and opioids.

Management of patients received at Kenyatta National Hospital was mainly supportive, and included Gastric lavage, emesis, respiratory support and diuresis. Patients admitted were given
intravenous fluids, activated charcoal, ranitidine injection and diuretics. Antidotes (atropine and pralidoxime) were only available for organophosphates.

Rodenticides remain the most common cause of suspected paediatric pesticide poisoning in the United Kingdom, accounting for 42% (Meredith, 1993). In the survey conducted at Kenyatta National Hospital, rodenticides account for 36% of all the cases of pesticide poisoning while organophosphates that account for 34% of the patients reviewed (Table 3). Amitraz accounts for 8% of the all the cases of pesticide poisoning at Kenyatta National Hospital (Figure 1).

The symptoms of Amitraz poisoning include CNS depression (drowsiness, coma and convulsions), respiratory depression, bradycardia, miosis, hypersalivation, nausea, vomiting and ataxia (Ellenhorn, 1997; Ulukaya, 2001). In the study, 44% (30/68) of the patients admitted with amitraz poisoning were received in the hospital comatose and required respiratory support and/or admission in the intensive care unit. Other symptoms observed included increased secretions, vomiting, abdominal pain, diarrhoea, respiratory depression, drowsiness and hypothermia.
Some of the symptoms of amitraz toxicity can be confused with signs and symptoms for other toxicants, including opioids and organophosphates (Yilmaz, 2003). In addition, many physicians assume that amitraz is an organophosphate and manage it as such. In the cases reviewed management of amitraz poisoning almost always included antidotal treatment with atropine and pralidoxime.

Amitraz is an alpha-2 adrenergic agonist. Therefore, drugs such as yohimbine which are alpha-2-adrenergic antagonists can be used to reverse the effects of amitraz poisoning.

In this study, the intraperitoneal median lethal dose (LD$_{50}$) for amitraz in rats was determined to be 75.01 mg/kg body weight. Co-administration of yohimbine and amitraz slightly increased the LD$_{50}$ to 78.11 mg/kg body weight, thus indicating a reduction in the relative toxicity of Amitraz. The increase in the median lethal dose, though marginal, was calculated to be statistically significant.

Alkalization of urine effectively increases the excretion of acidic toxicants and their metabolites.

Since amitraz produces an acid metabolite, this study investigated the effect of urinary alkalization on the relative toxicity of amitraz.
Co-administration of amitraz with Sodium bicarbonate, a urinary alkalinizer, increased the median lethal dose to 91.85 mg/kg body weight. This difference was demonstrated to be statistically significant using a t-test (p < 0.01). Hence, urinary alkalination with Sodium bicarbonate reduces the relative toxicity of amitraz in rats.
5.2 CONCLUSIONS

The following general conclusions can be made based on the findings of the present study:

1. Amitraz accounts for a significant proportion of poisoning among humans, constituting 8% of all the cases reviewed.

2. The intraperitoneal LD$_{50}$ of amitraz in rats is 75.01mg/kg body weight.

3. Yohimbine antagonism does marginally, though significantly increases the LD$_{50}$ of amitraz in rats and therefore reduces the relative toxicity of amitraz in rats.

4. Urinary alkalization with Sodium Bicarbonate significantly increases the LD$_{50}$ of amitraz in rats and therefore reduces the relative toxicity of Amitraz in rats.
5.3 RECOMMENDATIONS

The following general recommendations can be made based on the findings of this study:

1. In the absence of a specific antidote, supportive management and urinary alkalination should be used in management of amitraz poisoning in humans.

2. Physicians and other professionals involved in patient care need continuous updates on the toxicants frequently responsible for poisoning and their management to reduce the frequency of presumptive diagnosis and management.

3. Legal classification and commercial availability of products more commonly responsible for human poisoning need review. Such products should have limited distribution and where possible should be secured and stored in childproof packaging to reduce the incidences of poisoning.

4. There is need to further study the usefulness of both urinary alkalination and yohimbine antagonism in management of Amitraz poisoning in humans.
REFERENCES


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Appendix 1: Ethical approval obtained from the Ethics and Research Committee, Kenyatta National Hospital
Appendix 2: Data collection form used to obtain demographic information from patients admitted with pesticide poisoning in Kenyatta National Hospital

<table>
<thead>
<tr>
<th>CASE NO</th>
<th>AGE (yrs)</th>
<th>SEX</th>
<th>PLACE OF POISONING (RURAL OR URBAN)</th>
<th>LOCATION (HOME, WORK, SOCIAL)</th>
<th>SOCIO-ECONOMIC LEVEL (LOW, MIDDLE, HIGH)</th>
<th>IDENTITY OF TOXICANT</th>
<th>TYPE OF EXPOSURE (ACCIDENTAL, INTENTIONAL, UNDETERMINED)</th>
<th>ROUTE OF POISONING (ORAL, DERMAL, OTHER)</th>
<th>MAJOR PRESENTING CLINICAL SIGNS</th>
<th>LENGTH OF STAY</th>
<th>OUTCOME (CURED/DIED)</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
</tbody>
</table>
Appendix 3: Raw data of Acute Toxicity study following intraperitoneal administration of 113mg/kg Amitraz

**TOXICITY TESTING:** Median lethal dose: Amitraz 75mg/kg
Date: 30.03.06

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Sex</th>
<th>Weight (g)</th>
<th>Test compound</th>
<th>Dose (mg/kg)</th>
<th>Original Conc. (%)</th>
<th>Volume (ml)</th>
<th>Time given</th>
<th>Outcome</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>M</td>
<td>260</td>
<td>Amitraz</td>
<td>75</td>
<td>12.5</td>
<td>0.16</td>
<td>2.43 pm</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>173</td>
<td>Amitraz</td>
<td>75</td>
<td>12.5</td>
<td>0.10</td>
<td>2.45 pm</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>280</td>
<td>Amitraz</td>
<td>75</td>
<td>12.5</td>
<td>0.17</td>
<td>2.46 pm</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>258</td>
<td>Amitraz</td>
<td></td>
<td></td>
<td>0.16</td>
<td>2.48 pm</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>232</td>
<td>Amitraz</td>
<td></td>
<td></td>
<td>0.14</td>
<td>2.49 pm</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>262</td>
<td>Amitraz</td>
<td></td>
<td></td>
<td>0.16</td>
<td>2.52 pm</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>296</td>
<td>Amitraz</td>
<td></td>
<td></td>
<td>0.18</td>
<td>2.53 pm</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>223</td>
<td>Amitraz</td>
<td></td>
<td></td>
<td>0.13</td>
<td>2.56 pm</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>311</td>
<td>Amitraz</td>
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<td></td>
<td>0.19</td>
<td>2.57 pm</td>
<td>Dead</td>
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</tr>
<tr>
<td>10</td>
<td>M</td>
<td>291</td>
<td>Amitraz</td>
<td></td>
<td></td>
<td>0.17</td>
<td>2.58 pm</td>
<td>Dead</td>
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</tr>
</tbody>
</table>
APPENDIX 4: Raw data of Acute Toxicity study following intraperitoneal administration of amitraz and yohimbine

TOXICITY TESTING: Median lethal dose: Amitraz 170mg/kg + yohimbine 0.5mg/kg Date 12.04.06

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Sex</th>
<th>Weight (g)</th>
<th>Test compound</th>
<th>Dose (mg/kg)</th>
<th>Original Conc. (%)</th>
<th>Volume (ml)</th>
<th>Time given</th>
<th>Outcome</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>300</td>
<td>Amitraz + yohimbine</td>
<td>170 0.5 12.5 6.25</td>
<td>0.41 0.024</td>
<td>3.34 pm</td>
<td>dead</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>293</td>
<td>Amitraz + yohimbine</td>
<td>170 0.5 12.5 6.25</td>
<td>0.40 0.023</td>
<td>3.36 pm</td>
<td>dead</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>306</td>
<td>Amitraz + yohimbine</td>
<td>170 0.5 12.5 6.25</td>
<td>0.41 0.024</td>
<td>3.38 pm</td>
<td>dead</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>301</td>
<td>Amitraz + yohimbine</td>
<td>170 0.5 12.5 6.25</td>
<td>0.41 0.024</td>
<td>3.39 pm</td>
<td>Dead</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>266</td>
<td>Amitraz + yohimbine</td>
<td>170 0.5 12.5 6.25</td>
<td>0.36 0.021</td>
<td>3.41 pm</td>
<td>Dead</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>228</td>
<td>Amitraz + yohimbine</td>
<td>170 0.5 12.5 6.25</td>
<td>0.31 0.02</td>
<td>3.45 pm</td>
<td>Dead</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>201</td>
<td>Amitraz + yohimbine</td>
<td>170 0.5 12.5 6.25</td>
<td>0.27 0.02</td>
<td>3.47 pm</td>
<td>dead</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>274</td>
<td>Amitraz + yohimbine</td>
<td>170 0.5 12.5 6.25</td>
<td>0.37 0.02</td>
<td>3.50 pm</td>
<td>Dead</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>186</td>
<td>Amitraz + yohimbine</td>
<td>170 0.5 12.5 6.25</td>
<td>0.25 0.02</td>
<td>3.52 pm</td>
<td>Dead</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>241</td>
<td>Amitraz + yohimbine</td>
<td>170 0.5 12.5 6.25</td>
<td>0.33 0.02</td>
<td>3.56 pm</td>
<td>Dead</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX 5: Raw data of Acute Toxicity study following intraperitoneal administration of amitraz and Sodium bicarbonate

TOXICITY TESTING: Median lethal dose: Amitraz 50mg/kg + NaHCO₃ 50mg/kg  date 19.04.06

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Sex</th>
<th>Weight</th>
<th>Test compound</th>
<th>Dose (mg/kg)</th>
<th>Original Conc. (%)</th>
<th>Volume (ml)</th>
<th>Time given</th>
<th>Outcome</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>223</td>
<td>Amitraz + NaHCO₃</td>
<td>50 50</td>
<td>12.5 8.4</td>
<td>0.09 0.13</td>
<td>12.22pm</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>286</td>
<td>Amitraz + NaHCO₃</td>
<td>50 50</td>
<td>12.5 8.4</td>
<td>0.15 0.17</td>
<td>12.26pm</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>240</td>
<td>Amitraz + NaHCO₃</td>
<td>50 50</td>
<td>12.5 8.4</td>
<td>0.10 0.14</td>
<td>12.28pm</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>204</td>
<td>Amitraz + NaHCO₃</td>
<td>50 50</td>
<td>12.5 8.4</td>
<td>0.08 0.12</td>
<td>12.31pm</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>239</td>
<td>Amitraz + NaHCO₃</td>
<td>50 50</td>
<td>12.5 8.4</td>
<td>0.10 0.14</td>
<td>12.32pm</td>
<td>Alive</td>
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<tr>
<td>6</td>
<td>M</td>
<td>238</td>
<td>Amitraz + NaHCO₃</td>
<td>50 50</td>
<td>12.5 8.4</td>
<td>0.09 0.14</td>
<td>12.35pm</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>168</td>
<td>Amitraz + NaHCO₃</td>
<td>50 50</td>
<td>12.5 8.4</td>
<td>0.06 0.10</td>
<td>12.39pm</td>
<td>dead</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>237</td>
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<td>12.5 8.4</td>
<td>0.10 0.14</td>
<td>12.44pm</td>
<td>Dead</td>
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<tr>
<td>9</td>
<td>M</td>
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<td>50 50</td>
<td>12.5 8.4</td>
<td>0.14 0.20</td>
<td>12.53pm</td>
<td>Alive</td>
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</tr>
<tr>
<td>10</td>
<td>F</td>
<td>206</td>
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<td>50 50</td>
<td>12.5 8.4</td>
<td>0.08 0.12</td>
<td>12.55pm</td>
<td>Alive</td>
<td></td>
</tr>
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</table>
APPENDIX 5: Raw data of Acute Toxicity study following intraperitoneal administration of amitraz and Sodium bicarbonate

**TOXICITY TESTING:** Median lethal dose: Amitraz 50mg/kg + NaHCO₃ 50mg/kg ___date_19.04.06___

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Sex</th>
<th>Weight (g)</th>
<th>Test compound</th>
<th>Dose (mg/kg)</th>
<th>Original Conc. (%)</th>
<th>Volume (ml)</th>
<th>Time given</th>
<th>Outcome</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>223</td>
<td>Amitraz +NaHCO₃</td>
<td>50</td>
<td>12.5</td>
<td>0.09</td>
<td>12.22pm</td>
<td>Alive</td>
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<tr>
<td>2</td>
<td>M</td>
<td>286</td>
<td>Amitraz +NaHCO₃</td>
<td>50</td>
<td>12.5</td>
<td>0.15</td>
<td>12.26pm</td>
<td>Alive</td>
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</tr>
<tr>
<td>3</td>
<td>M</td>
<td>240</td>
<td>Amitraz +NaHCO₃</td>
<td>50</td>
<td>12.5</td>
<td>0.10</td>
<td>12.28pm</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>204</td>
<td>Amitraz +NaHCO₃</td>
<td>50</td>
<td>12.5</td>
<td>0.08</td>
<td>12.31pm</td>
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</tr>
<tr>
<td>5</td>
<td>M</td>
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