A SURVEY OF ALDICARB POISONING IN DOGS AND CATS IN GAUTENG AND EVALUATION OF THE EFFICACY OF HYDROXYPROPYL-β-CYCLODEXTRIN AS A TREATMENT IN ALDICARB POISONING

By

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Submitted in partial fulfilment of the requirements for the degree Magister Scientiae in the Department of Paraclinical Sciences in the Faculty of Veterinary Science, University of Pretoria

Date submitted: January 2005-

ACKNOWLEDGEMENTS

The promoters, Prof. Botha and Dr. Naidoo, are especially thanked for their guidance. I also thank Dr. van Sckalkwyk, who created the geographical (GIS) maps of Gauteng. The financial contributions received from Bayer Crop Science and the Faculty of Veterinary Science is gratefully acknowledged. I also appreciate the assistance received from Mr. Smuts and Mr. Selahle of the University of Pretoria Biomedical Research Centre (UPBRC). Mrs. Mülders is thanked for technical assistance and Miss Bekker for drawing the chemical structures of hydroxypropyl-β-cyclodextrin and aldicarb. The contribution of the private veterinarians, who completed the questionnaires, is highly appreciated.

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A SURVEY OF ALDICARB POISONING IN DOGS AND CATS IN GAUTENG AND EVALUATION OF THE EFFICACY OF HYDROXYPROPYL-β-CYCLODEXTRIN AS A TREATMENT IN ALDICARB POISONING

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Worldwide, pesticides are applied to protect crops against insects, fungi and other parasites. Without these chemicals it would not be possible to produce sufficient food to satisfy the demand of an ever-increasing world population. Unfortunately, many cases of accidental and intentional poisoning of humans and animals occur and the objectives of this study were to obtain statistics of aldicarb poisoning in companion animals in Gauteng Province and to evaluate hydroxypropyl-β-cyclodextrin as a potential treatment.

Cyclodextrins are ring-shaped oligosaccharides with a hydrophilic exterior and a hydrophobic interior. The interior cavity is capable of complexing fat-soluble molecules small enough to fit inside. Aldicarb is moderately lipid-soluble, non-ionized and of low molecular weight and thus fits all criteria for complexation with cyclodextrin.

Questionnaires were posted to all private practitioners in Gauteng. The survey was designed to determine the percentage of aldicarb cases seen, clinical signs observed, treatment regimen, proposals for preventative actions and more effective treatments. Other questions included duration of treatment, survival rate, cost to client, post-mortem findings and reasons for poisonings. Thirty-four percent of respondants indicated the total number of all clinical cases presented at their practices during 2003. The percentage of suspected aldicarb cases as a proportion of all cases ranged from 0.05 - 2.6 % for dogs and 0.09 - 3.33 % for cats. Only 26.5 % of practitioners sometimes submitted samples for laboratory confirmation of aldicarb poisoning.

Salivation and tremors were the most common clinical signs observed by private practitioners and the majority of suspected poisoning cases were treated with atropine, intravenous fluid and electrolyte therapy and the oral administration of activated charcoal.

Thirty-three respondents thought that there was an increase in the number of aldicarb cases, but 35 felt there was no increase during 2003. Fifteen respondents were reluctant to venture an opinion. Most veterinarians indicated that criminal intent was the main reason why animals were poisoned and 95 % of respondents reported that it occurred throughout the year, but an increased incidence was observed during holiday periods.Survival times in the majority of rats dosed with aldicarb and receiving intravenous cyclodextrin were longer, compared to the control rats only dosed with aldicarb per os.

Rats receiving cyclodextrin immediately before aldicarb, survived longer when compared to rats, which received aldicarb prior to cyclodextrin.

Key words: aldicarb, cats, dogs, Gauteng, hydroxypropyl-β-cyclodextrin, poisoning, questionnaire, rats, survey, treatment.

SAMEVATTING

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Plaagdoders word wêreld-wyd gebruik om gesaaides teen insekte, swamme en ander parasiete te beskerm. Sonder hierdie chemikalieë, sou dit nie moontlik gewees het om genoeg voedsel te produseer vir ‘n groeiende wêreld bevolking nie.

Heelwat vergiftigings van mens en dier kom gereeld voor en kan kwaadwillig of per ongeluk geskied. Die doelwitte van die studie was om statistieke van aldikarbvergiftiging in troeteldiere in Gauteng Provinsie te bekom en hidroksiepropiel-β-siklodekstrien as ‘n moontlike bindingsagent vir aldikarb te evalueer.

Siklodekstriene is ringvormige oligosakkariede met ‘n hidrofiliese buitekant en ‘n hidrofobiese binnekant. Die binneste holte het die vermoë om vetoplosbare molekules, wat klein genoeg is, te bind. Aldikarb is matig lipied-oplosbaar,

nie-geioniseerd en het ‘n lae molekulêre massa en voldoen aan al die vereistes vir kompleksering met siklodekstriene.

Vraelyste is na al die veearts-praktyke in Gauteng gepos met die doel om vas te stel watter persentasie van alle kliniese gevalle in 2003 as aldikarb vergiftiging gediagnoseer was, welke kliniese tekens die meeste voorkom, watter behandeling tans in gebruik is en voorstelle vir die voorkoming van vergiftiging en meer effektiewe behandeling protokolle. Ander inligting wat verlang is, was die tydsduur van behandeling, pasiënt oorlewings persentasie, veeartseny kostes, nadoodse bevindinge en redes vir vergiftiging.

Vier-en-dertig persent van veeartse het die totale aantal kliniese gevalle in 2003 aangedui. Die persentasie van verdagte aldikarb gevalle as ‘n proporsie van alle gevalle het van 0.05 - 2.6 % in honde en 0.09 - 3.33 % in katte gewissel. Slegs 26.5 % respondente het somtyds monsters na ‘n laboratorium ingestuur om aldikarbvergiftiging te bevestig.

Speekselvloei en spiertrillings was die mees algemene kliniese tekens wat waargeneem was en die meeste van die verdagte gevalle is met atropien, vloeistof- en elektroliet terapie asook met orale geaktiveerde houtskool behandel.

Drie-en-dertig kollegas het geskat dat daar gedurende 2003 ‘n toename in aldikarbvergiftiging was, terwyl 35 van mening was dat hulle nie meer gevalle as vroeër gesien het nie. Vyftien praktisyns was onseker en het nie ‘n

antwoord verskaf nie. Die meerderheid veeartse was van mening dat kriminele redes die hoofoorsaak van vergiftiging is. Geen seisoenale voorkoms was duidelik nie en 95 % van die respondente het gevalle dwarsdeur die jaar gesien, alhoewel die insidensie gedurende vakansie-tye toeneem.

Die meerderheid rotte wat intraveneuse siklodekstrien en mondelinge aldikarb toegedien is, het langer oorleef as kontrole rotte wat slegs orale aldikarb ontvang het. Die rotte wat siklodekstrien onmiddellik voor aldikarb ontvang het, het langer geleef as rotte wat eers met aldikarb gedoseer is en siklodekstrien onmiddellik daarna toegedien is.

Sleutelwoorde: aldikarb, behandeling, Gauteng, hidroksiepropiel-β-siklodekstrien, honde, katte, opname, rotte, vergiftiging, vraelys.

CHAPTER 1

INTRODUCTION

Pesticides are frequently and widely used to protect crops from various pests. Annually, 500 000 tonnes are produced in United States of America (USA), representing over 900 compounds, which are formulated into 25 000 registered products and distributed worldwide (Fikes 1990). The USA Environmental Protection Agency (EPA) estimated that pesticides were used on more than 900 000 farms and in 69 million households in 1993 (Talcott & Dorman 1997). An estimated 281.5 million kilograms of herbicides, 112.1 million kilograms of insecticides, 59.5 million kilograms of fungicides and 37.7 million kilograms of other pesticides are used annually (Talcott & Dorman 1997).

Without these chemicals, a significant percentage of the crops will be lost. Although the benefits regarding protection of food sources are obvious, the use of pesticides, unfortunately, results in poisonings in many animals and humans every year. In 1990, the American Association of Poison Control Centres received 41 854 calls regarding exposure of animals to poisons. Pesticide exposure was reported in 29.6 % of the calls received (Talcott & Dorman 1997).

In South Africa, data of confirmed cases of aldicarb mortalities and/or exposure, obtained from the Toxicology Division, Onderstepoort Veterinary Institute (OVI), indicate the following: 72 cases were confirmed during 1998,

67 cases in 1999 and 72 cases were recorded in 2000; this increased to 115 in 2001 and 114 in 2002 and decreased somewhat to 97 in 2003 (J P J Joubert, Onderstepoort Veterinary Institute, unpublished data).

Organophosphors are often implicated as a cause of serious intoxication in humans, where children, farmers and farm labourers are mostly affected (Haddad 1983 (a)). Animals are usually poisoned by dipping and accidental ingestion. Annually, more than 100 million kilograms of organophosphors are produced worldwide (Barret *et al.* 1985). Outbreaks of poisoning occur in numerous countries. One farmer lost 13 out of 19 beef heifers from organophosphor poisoning in Iowa, USA (Casteel & Carson 1984). In 1968, 6278 sheep became ill in Utah, USA after eating forage contaminated with an organophosphate compound. Of those affected 4500 died or had to be euthanased (Van Kampen *et al*. 1969). Outbreaks of organophosphor poisoning in animals are frequent occurrences, but are usually not published.

During 2003, several cases of organophosphor poisoning were confirmed in South Africa, including 13 cases of monocrotophos, 6 cases each of terbuphos and diazinon and 5 cases of parathion (J PJ Joubert OVI, unpublished data). A few cases involving several other organophosphor compounds were also confirmed.

1.1. Problem statement

Accidental and intentional toxicoses occur frequently, therefore, the only two options available to decrease the incidence of poisoning and subsequent

mortalities associated with toxicosis, are to install more effective preventative measures and/or to evaluate other therapeutic modalities.

1.2. Objectives

The aims of this project were:

- To ascertain the actual incidence of aldicarb poisoning in Gauteng.
- To evaluate the efficacy of hydroxypropyl-β-cyclodextrin as a binding agent for aldicarb poisoning.

CHAPTER 2

LITERATURE REVIEW

2.1. Pesticides

Pesticides represent a wide and diverse group of compounds, including insecticides, herbicides, rodenticides, fungicides and nematocides. Several agricultural catastrophes in the past have focussed research on the use of chemical control agents, leading to the development of pesticides. During 1845, the potato crop in Ireland was destroyed by *Phytophthora infestans*, causing famine and forcing 1,5 million people to emigrate (Cabras 2003). In 1878, French vineyards were destroyed by downy mildew introduced from the United States of America (USA).

Sulphur and arsenic were some of the first pesticides in use from approximately 1000 BC. It was only in 1939 that the first organic pesticide, dichlorodiphenyltrichloroethane (DDT) was introduced onto the market (Cabras 2003).

2.2. Classification of insecticides and acaricides

2.2.1. Botanical insecticides

(a) Nicotine and nicotinoids

The main compounds are nicotine, nornicotine and anabasine of which nicotine is the most widely used and the most potent (Kamrin 1997; Matsumura 1980). Commercially, nicotine is obtained from *Nicotiana tabacum* and *N. rustica* (Matsumura 1980). Imidacloprid ("*Advantage*") is a new synthetic compound in this group (Stevens & Breckenridge 2001).

These chemicals initially activate and then inhibit the nicotinic cholinergic receptors at the nerve ganglia and synapses, which lead to neural excitation followed by paralysis and death of the insect (Kamrin 1997; Matsumura 1980; Stevens & Breckenridge 2001).

(b) Rotenone and rotenoids

Six rotenone derivatives occur naturally of which rotenone is the most potent (Matsumura 1980). Rotenone and rotenoids are extracted from *Derris elliptica, D. malaccensis*, *Lonchocarpus utlis and L. urucu,* which are all members of the Fabaceae family (Marrs 1993). Rotenoids are highly toxic to fish (Brander *et al*. 1991; Kamrin 1997; Matsumura 1980) and some insects, but only moderately toxic to mammals (Kamrin 1997). The mammalian toxicity varies greatly between animal species and route of administration (Matsumura 1980). Rotenone is an inhibitor of aerobic metabolism and also results in nerve conduction disturbances and paralysis of the insect (Matsumura 1980).

(c) Pyrethrum and pyrethroids

This is the largest group of plant-derived insecticides. Pyrethrum is isolated from the flowers of *Chrysanthemum* species, especially *Chrysanthemum cinerariaefolium* and *C. coccineum* (Kamrin 1997; Matsumura 1980). Pyrethrum is essentially non-toxic to mammals, but a rapid-acting insecticide (Matsumura 1980). There are over twenty natural compounds, (Kamrin 1997) which are contact insecticides and examples of synthetic pyrethroid are

permethrin, cypermethrin and fenvalerate (Brander *et al*. 1991). Pyrethroids kill insects by severely disrupting nerve function (Matsumura 1980; Stevens & Breckenridge 2001).

2.2.2. Avermectins

The avermectins, e.g. abamectin and ivermectin (*Ivomec*), are a group of macrocyclic lactones isolated from the soil fungus *Streptomyces avermitilis* (Kamrin 1997; Stevens & Breckenridge 2001). The avermectins stimulate the release of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) at synapses. GABA released from the presynaptic nerve terminal binds to a postsynaptic GABA receptor which opens chloride channels and promotes chloride ion influx into the postsynaptic neuron, which hyperpolarizes the membrane, thereby dampening (inhibitory effect) nerve impulse conductance (Stevens & Breckenridge 2001). In insects it leads to paralysis and in mammals avermectin toxicity is characterised by ataxia and coma-like sedation (Stevens & Breckenridge 2001).

2.2.3. Organochlorines (chlorinated hydrocarbons)

Chlorinated hydrocarbons, especially DDT, were widely used from the 1940's to the 1960's for the control of agricultural pests and *Anopheles* (malaria-transmitting) mosquitoes (Kamrin 1997). Since then, their use has been curtailed or banned because of their persistence in the environment (Kamrin 1997) and tendency to biomagnify in the food chain (Cabras 2003; Timbrell 1997).

Three classes exist namely:

- Diphenylethanes e.g. methoxychlor and DDT
- Cyclodienes e.g. dieldrin, endrin, heptachlor and toxaphene
- Cyclohexanes e.g. hexachlorocyclohexane (HCH) (Cabras 2003; Marrs 1993).

In both insects and animals these chloride-channel blocking insecticides disrupt the resting transmembrane potential inducing neural excitation followed by paralysis and death (Brander *et al*. 1991; Kamrin 1997; Matsumura 1980; Stevens & Breckenridge 2001).

The toxic effect of cyclodienes has also been ascribed to antagonism of GABA-receptors (Marrs 1993; Stevens & Breckenridge 2001). In addition, these compounds inhibit brain and heart calmodulin-regulated calcium pump activity (Marrs 1993). Gamma-benzenehexachloride (γ-BHC) probably also affects insects by interfering with their inositol metabolism (Brander *et al*. 1991).

2.2.4. Phenylpyrazoles

Fipronil ("*Frontline*") inhibits GABA-regulated chloride ion influx into nerve cells (Boothe 2001; Page 2002; Plumb 1999). Binding affinity to mammalian receptors are much lower, thus it is relatively safe for dogs and cats (Page 2002). Fipronil is not significantly absorbed from the skin, but translocate dermally to the lipids of hair follicles and sebaceous glands and continues to be released over time, resulting in a long residual activity (Page 2002; Plumb 1999).

2.2.5. Insect growth regulators

(a) Juvenile hormone mimics

Examples are diofenolan, pymetrozine ("*Sterling*") pyriproxifen ("*Knack*") (Stevens & Breckenridge 2001) and methoprene ("*Ovitrol*") (Hodgson & Levi 1987). Exposure to these analogues at moulting results in mixed larval/pupal or larval/adult morphologies. It is most effective in the last larval or early pupal stages (Stevens & Breckenridge 2001).

(b) Larvicides

Clofentezine, cyromazine ("*Vetrazin*") and hexythiazox are examples of compounds killing the developing larvae (Stevens & Breckenridge 2001).

(c) Moult accelerators

Tebufenozide cause the larvae to stop feeding and to moult successfully (Stevens & Breckenridge 2001). These compounds are also referred to as ecdysone agonists.

(d) Moult inhibitors

Benzoylureas, including diflubenzuron and teflubenzuron (Stevens & Breckenridge 2001; Hodgson & Levi 1987), prevent chitin synthesis in insects. In the absence of chitin, the cuticle is unable to support the insect (Stevens & Breckenridge 2001).

Lufenuron ("*Program*") belongs to the benzoylphenylureas and inhibit chitin synthase (Page 2002), thereby disrupting polymerization and deposition of chitin (Boothe 2001; Page 2002; Plumb 1999). The drug is absorbed from the small intestine and stored in the adipose tissue of the host, which acts as a reservoir to distribute drug slowly into the circulation (Boothe 2001; Plumb 1999).

2.2.6. Pheromones

Pheromones are chemical sex attractants. In the USA, 17 arthropod – and 11 lepidopteran pheromones have been registered. The major lepidopteran pheromone is tetradecenyl acetate (Stevens & Breckenridge 2001).

2.2.7. Thiocyanates

Thiocyanates halt electron transfer and cellular respiration and produce rapid knockdown and paralysis of insects. Examples of trade names include "*Lethane 60*" and "*Thanite*" (Matsumura 1980).

2.2.8. Dinitrophenols

These substances uncouple oxidative phosphorylation and specifically act on Coenzyme Q or the Cytochrome b-c_1 complex. The electron transport system functions normally, but the production of ATP is uncoupled from the electron transport process. Oxygen consumption increases, but no ATP is formed (Stevens & Breckenridge 2001). These compounds are quite toxic to cells of all types, including plant cells. They will kill mite eggs and are therefore useful

as dormant sprays in winter. Examples include dinitrocresol and dinitrocyclohexylphenol (Matsumura 1980).

2.2.9. Fluoroacetate derivates

Insecticidal action is as result of fluoroacetate, converting *in vivo* to fluorocitrate. The enzyme aconitase in the Krebs cycle is inhibited, halting ATP production. Examples include fluoroacetic acid and fluoroacetamide (Matsumura 1980).

2.2.10. Sulphonates, sulphones and sulphides

These are acaricidal compounds that contain two chlorinated benzene rings. Examples include "*Ovotran*" (only ovicide) and "*Sulphenone*" (Matsumura 1980).

2.2.11. Fumigants

Fumigants are extremely volatile substances and are useful to prevent insects from damaging stored products. Examples include methyl bromide and ethylene dichloride (Matsumura 1980).

2.2.12. Synergists

The majority of synergists contain an active moiety, namely a methylene-dioxyphenyl group (Matsumura 1980). The synergists inhibit the insect's mixed-function oxidases (MFO's) and it is unable to detoxify insecticides. The toxicity of certain insecticides can be significantly increased by the addition of a synergist, which might not be insecticidal on its own (Matsumura 1980).

Examples include sesamin (Matsumura 1980) and piperonyl butoxide (Hodgson & Levi1987; Matsumura 1980).

2.2.13. Triazepentadienes

These substances are also known as formamidines, e.g. amitraz ("*Triatix*"), which is effective against ticks, lice and mange mites (Brander *et al*. 1991). Amitraz acts as a reversible α_2-adrenergic agonist, both in the central and peripheral nervous system (Blodgett 2002; Booth & McDonald1988; Plumb 1999). *In vitro* experiments suggest inhibition of monoamine oxidase (MAO), but no significant effect was shown to occur *in vivo* (Blodgett 2002). Idiosyncratic toxic reactions may occur in Chihuahuas and Miniature Poodles. Amitraz is toxic to cats and contra-indicated in horses as it reduces intestinal motility, with resultant impaction of the large colon (Baggot 2001).

2.2.14. Chlordimeform and analogues

The acaricide and insecticide chlordimeform ("*Galecron*") acts as a monoamine oxidase (MAO) inhibitor. In addition, this compound also increases serotonin and noradrenalin concentrations in rat brain and may also uncouple oxidative phosphorylation (Matsumura 1980).

2.2.15. Endotoxins

Bacillus thuringiencis is an aerobic, spore-forming, Gram-positive, rod-shaped bacterium. At sporulation, it forms an inclusion body that contains insecticidal protein toxins (Kamrin 1997; Stevens & Breckenridge 2001) e.g. thuricide (Hodgson & Levi 1987). When consumed by insects, the inclusion body is

dissolved in the insect's intestinal tract and ε-endotoxins are released, which are then cleaved to the active form by proteases (Stevens & Breckenridge 2001). The active endotoxins bind to midgut epithelial cell membranes and alter their ion permeability properties by forming a cation channel. Ion movements through this pore disrupt potassium and pH gradients (Stevens & Breckenridge 2001). The insect stops eating, as digestion is interrupted because there is lysis of the epithelium as well as gut paralysis, which results in death (Kamrin 1997; Stevens & Breckenridge 2001). These preparations have been available for over 30 years (Stevens & Breckenridge 2001).

2.2.16. Organophosphors

The organophosphors are very popular pesticides, as they are highly effective and disintegrate into harmless radicals within days of application (Haddad 1983 (a)). These compounds do not persist in body tissues and the environment as compared to DDT and other organochlorine pesticides (Chambers & Carr 2002; Haddad 1983 (a)).

This pesticide is the most widely marketed insecticide, with a market share of 37.2 % of all compounds sold (Cabras 2003). The basic molecule consists of a central phosphorus atom linked to numerous side-chains (Kamrin 1997). Various subgroups of organophosphors are recognized.

(a) Pyrophosphates

Tetraethylpyrophosphate (TEPP) is a highly poisonous compound with an oral LD_{50} in male rats of 1 mg/kg. Hydrolysis of TEPP releases phosphoric acid, which is highly corrosive (Matsumura 1980).

(b) Phosphorohalides and cyanides

This subgroup contains the “nerve gases” e.g. tabun (Matsumura 1980).

(c) Dialkylarylphoshates, phosphorothioates and phosphorodithioates

The phosphorothioates are inactive compounds, which require bioactivation by Cytochrome P-450 to form the toxic compound (Chambers & Carr 2002; Marrs 1993; Mosha 1993; Taylor 1996). The phosphorus-sulphur (P=S) bond is converted by oxidative desulphuration to form the phosphorus-oxygen (P=O) metabolite, e.g. parathion is converted to paraoxon (Chambers & Carr 2002; Mosha 1993). Other examples within these classes include fenchlorphos, fenthion, coumaphos and diazinon.

(d) Trialkylphosphates and thiophosphates

Examples in this subgroup include trichlorfon, dichlorvos, demeton and malathion. The latter is one of the safest insecticides, due to the presence of carboxyl groups, which are hydrolysed by mammals. Malathion kills insects by contact or vapour action and is also a stomach poison (Matsumura 1980).

2.2.17.

Carbamates

Carbamates were first identified in the extracts of the Calabar bean, containing physostigmine, which is a methylcarbamate ester (Kamrin 1997). There are also different carbamate subgroups.

(a) Naphthylcarbamates

Carbaryl ("*Karbadip spray*") is a broad-spectrum insecticide, which controls 100 - 150 different insects species. It has a low mammalian toxicity, with an acute oral LD_{50} in rats of 500 - 700 mg/kg (Matsumura 1980).

(b) Phenylcarbamates

Mexacarbate ("*Zectran*") kills a broad spectrum of insects and certain molluscs. It is highly toxic, with an acute oral LD_{50} in rats of 15 - 63 mg/kg (Matsumura 1980). Propoxur or arprocarb ("*Baygon*") is safer with an acute oral LD_{50} in rats of 95 - 104 mg/kg (Matsumura 1980).

(c) Heterocyclic dimethylcarbamates

Pyrolan is moderately toxic, with an oral LD_{50} in rats of 90 mg/kg (Matsumura 1980). Isolan is more toxic to mammals than pyrolan and has a better systemic effect. Both compounds have a short residual half-life and can be absorbed directly through leaves and roots of plants in quantities toxic to aphids and thrips. Dimetilan is used to impregnate plastic fabric e.g. fly strips (Matsumura 1980).

(d) Heterocyclic methylcarbamates

Carbofuran ("*Curaterr*; *Furadan*") is highly toxic, with an acute oral LD_{50} in rats of 5 mg/kg (Matsumura 1980).

(e) Thio- and dithiocarbamates

Thiocarbamates have one sulphur atom substituting an oxygen atom, while dithiocarbamates have two sulphur atoms replacing oxygen atoms (Kamrin 1997). Thiocarbamates e.g. butylate, cartap and fenothiocarb inhibit the enzyme acetylcholinesterase directly, dithiocarbamates e.g. ferbam, anobam and mancozeb, on the other hand, affect the nervous system through their main metabolite, carbon disulfide. These compounds affect the ability of the nerve cell to effectively conduct nerve impulses by altering the permeability of the nerve cell membrane and myelin sheath (Kamrin 1997).

(f) Oximes

Aldicarb ("*Temik*") is the most toxic carbamate with an acute oral LD_{50} in rats of 0.93 mg/kg (Tomlin 1994). "*Temik*" (Figure 1) is used as an insecticide, acaricide and nematocide.

Figure 1 *"Temik"* granules

Aldicarb (Figure 2) is also highly toxic to birds, where the oral LD_{50} range from 1.78 mg/kg in the red-winged blackbird to 5.34 mg/kg in the ring-necked pheasant, but is only moderately toxic to fish (Kamrin 1997). Low doses of aldicarb had no chronic, reproductive, teratogenic, mutagenic or carcinogenic effects (Kamrin 1997).

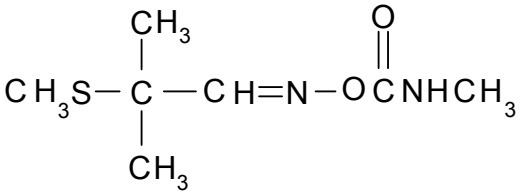

Figure 2 Chemical structure of aldicarb

This carbamate is moderately persistent, soluble and mobile in soil, especially sandy and sandy loam soils. In surface water, bacteria, sunlight and other reactions within the water rapidly destroy aldicarb and the half-life is 5 to 10 days, but in underground water the half-life can be months. In plants, aldicarb

is rapidly converted to a sulphoxide and more slowly to the sulphone metabolite (Kamrin 1997; Tomlin 1994).

In rats, dogs and cows, aldicarb is absorbed rapidly and completely. It is initially metabolised to sulphoxide and sulphone metabolites, which then undergo further metabolism. More than 80 % is excreted in the urine within 24 hours and more than 96 % within 3 - 4 days (Tomlin 1994).

2.3. Uses of organophosphors and carbamates

2.3.1. Veterinary uses

The organophosphors are used for the control of both internal - and external parasites. Oral dichlorvos is used for the control of *Gasterophilus* larvae in horses (Brander *et al*. 1991; Kamrin 1997). Various organophosphor ectoparasiticide formulations are utilized to control flies, mosquitoes, keds, ticks and lice (Brander *et al*. 1991; Matsumura 1980).

Carbamates are only used to control external parasites on production and companion animals. Carbaryl dust powders have achieved the widest usage and it is effective against ticks, lice, fleas and keds (Brander *et al*. 1991).

2.3.2. Agricultural uses

Organophosphors and carbamates are widely used to protect cotton, maize, wheat and a variety of other crops against insects (Kamrin 1997). Aldicarb is also used to control mites, nematodes and aphids in cotton, peanut and soybean crops (Kamrin 1997).

2.3.3. Chemical warfare

In 1936 - 1937 German chemists, led by Gerhard Schrader, synthesized three nerve gases namely sarin, tabun and soman for use against enemy troops (Aaron & Howland 1994; Chambers & Carr 2002). A fourth agent VX was developed in England a decade later (Aaron & Howland 1994). These four agents are extremely potent and rapid - acting, with death occurring within minutes of exposure (Aaron & Howland 1994).

2.3.4. Malicious poisoning

Aldicarb has often been implicated in malicious poisoning of dogs for criminal intent, as baits are easily prepared (Figure 3).

Figure 3 Baits laced with *"Temik"* granules

Both agricultural and veterinary pesticides are controlled by the Fertilizers, Farm Feeds, Agricultural and Stock Remedies Act (Act 36 of 1947), which stipulates procedures for distribution and usage by authorised persons. Even though these products are strictly controlled, their illegal use for the intentional poisoning of animals, especially dogs, occurs frequently (Figure 4).

Figure 4 *"Temik"* granules sold illegally as a rodenticide

2.4. Factors influencing the severity of toxicoses

The severity of organophosphor - and carbamate toxicoses can be influenced by a variety of different factors.

2.4.1. Animal factors

(a) Species differences

Parathion, administered orally, is more toxic to goats than to cows (Abdelsalam 1987 (b)). Horses are relatively sensitive to the organophosphor trichlorfon, which has a narrow margin of safety in this species (Gingerich 1981).

(b) Breed and phenotype

Brahman cattle (*Bos indicus*) are more sensitive to famphur and dioxathion than some European breeds (*Bos taurus*) (Abdelsalam 1987(b)). Sheep breeds differ in their ability to hydrolyze haloxon, with variation in the LD_{50} between susceptible and the more resistant phenotypes (Baker *et al*. 1980), even though haloxon is safe in sheep when administered at the recommended dose rates (Kingsbury & Curr 1967). Pure-bred Greyhounds are more sensitive to dichlorvos than cross-bred dogs (Snow & Watson 1973).

(c) Gender

Male rats are more sensitive than females to fenthion (Abdelsalam 1987 (b)). Hormone concentrations have an influence on toxicity as illustrated by the higher sensitivity of Holstein bulls with high testosterone levels to the effects of chlorpyrifos compared to Holstein steers (Haas *et al*. 1976). Female dogs are more sensitive to parathion compared to male dogs (Snow & Watson 1973).

(d) Age

Very young animals have inadequate capacity to detoxify and eliminate drugs while older animals may be weak and debilitated, with lowered resistance (Abdelsalam 1987(b); Meerdink 1989).

(e) Nutritional status

Protein deficiency decreases the liver microsomal enzyme activity and animals are more susceptible to the toxic effects (Abdelsalam 1987(b)).

(f) Disease

An animal with liver or kidney disease will be more susceptible to poisoning than a healthy animal, as the liver metabolizes toxins and the kidney excrete these biotransformed substances. Any other concomitant viral or bacterial infection will also lower the resistance of the animals (Abdelsalam 1987(b)).

2.4.2. Physiochemical characteristics

(a) Lipid solubility

Organophosphors and carbamates are mostly lipid soluble and are readily absorbed from most routes. Lipid soluble compounds are able to pass across biological membranes by dissolution in the phospholipids and movement down the concentration gradient (Timbrell 1997).

(b) Degree of ionisation

Only non-ionized drugs will cross biological membranes. An acidic drug is ionised in an alkaline environment and the same applies for a basic drug in an acidic environment (Timbrell 1997).

(c) Molecular size

Smaller molecules can pass through membranes easier than larger molecules. Molecules can move through membranes by filtration, passive diffusion, active transport, facilitated diffusion, phagocytosis or pinocytosis (Timbrell 1997).

(d) Plasma binding

Drugs and toxins differ in their affinities to bind to plasma proteins. Only the free drug/toxin can have a biochemical effect, whereas the bound compound is inactive (Timbrell 1997).

2.4.3. Pharmacodynamic interactions

Chemicals such as imidocarb can increase the toxicity of organophosphors, as they also inhibit acetylcholinesterase (Booth 1988; Plumb 1999).

2.5. Toxicokinetics

2.5.1. Absorption and distribution

The high lipid-solubility is advantageous for rapid absorption via the different routes. Aldicarb is rapidly absorbed following ingestion, because it is both moderately lipid-soluble and water-soluble. Solubility in water is 4.93 g/l, while it is soluble in most organic solvents, e.g. acetone, benzene and xylene (Tomlin 1994).

2.5.2. Biotransformation and excretion

In mammals xenobiotics are mainly biotransformed by the liver and excreted in the urine. Insects do not have these metabolizing enzymes in sufficient quantities to inactivate insecticides, which explains their sensitivity (Brander *et al*. 1991).

Both the organophosphors and carbamates are biotransformed by Phase 1 reactions (oxidation, reduction and hydrolysis) and Phase 2 reactions

(conjugation with glutathione, glycine and glucuronic acid). The end products are more water-soluble and easier excreted via urine (Ecobichon 2001).

Carbamates are detoxified through esterase-mediated hydrolysis or Cytochrome P-450-mediated oxidation (Chambers & Carr 2002). Organophosphor compounds can undergo simultaneous enzymatic attack at a number of different sites of the molecule, for example oxidative dealkylation, hydrolytic deoxyarylation, demethylation, dearylation, oxidative deethylation and deamination (Ecobichon 2001; Mosha 1993).

2.6. Mechanism of action of organophosphors and carbamates

2.6.1. Physiology of acetylcholine

Acetylcholine (Ach) acts as the neurotransmitter between nerve footplates and innervated cells of autonomic ganglia, the adrenal medulla, parasympathetic neuroeffector junctions, some sympathetic neuroeffector junctions, somatic neuromuscular junctions and certain regions of the central nervous system (Adams 1988; Brown & Taylor 1996; Mosha 1993). Acetylcholine produces excitation in some tissues e.g. smooth muscle of the gastrointestinal tract, but causes inhibitory responses in other tissues e.g. myocardium (Adams 1988). The excitatory effects are ascribed to depolarisation of the postsynaptic membrane, characterized by an increase in permeability of the membrane to both Na^+ and K^+ ions. On the other hand, inhibitory effects are due to hyperpolarization of the membrane caused by a selective increase in membrane permeability to K^+, but not Na^+ (Adams 1988). Acetylcholine is able to combine with the esterophilic and anionic sites of both

muscarinic and nicotinic receptors due to its molecular structure (Adams 1988).

2.6.2. Metabolism of acetylcholine

Acetylcholinesterase, the enzyme that hydrolyzes acetylcholine to acetic acid and choline, regulates acetylcholine concentrations at synapses (Haddad 1983(a)). Acetylcholine binds to two sites of acetylcholinesterase, with the quaternary nitrogen of Ach attaching to the anionic site and the carbonyl of the ester binding at the serine residue in the esteratic site, with subsequent loss of choline followed by rapid hydrolysis of the acetate from the serine residue (Chambers & Carr 2002; Mosha 1993).

2.6.3. Pathophysiology of organophosphors

The organophosphor‘s phosphate radical binds to the active site (the serine hydroxyl group) of acetylcholinesterase (Haddad 1983(a); Stevens & Breckenridge 2001). This binding is considered irreversible as after a period of time aging (caused by dealkylation of the organophosphorus moiety on the inhibited enzyme) occurs (Hansen 1995; Mosha 1993). Phosphorylated enzymes are inactive and unable to hydrolyze Ach at the synaptic - and myoneural junctions (Haddad 1983(a)). Plasma cholinesterase activity takes 4 - 6 weeks to return to baseline levels and erythrocyte acetylcholinesterase activity may take up to 5 - 7 weeks (Aaron & Howland 1987).

Although acetylcholinesterase inhibition is the main cause of intoxication, other mechanisms have also been described. The administration of

diisopropyl fluorophosphate significantly raised central nervous system dopamine levels in rabbits, which in turn also caused an increase in Ach concentrations in the thalamus and hypothalamus (Ho & Hoskins 1987). Some organophosphor agents that do not inhibit acetylcholinesterase are believed to cause their effects by altering or antagonizing the function of GABA, resulting in convulsions and death (Barret *et al*. 1985; Ho & Hoskins 1987).

A sub-acute syndrome, referred to as organophosphor-induced delayed neuropathy (OPIDN) may occur 7 - 14 days after exposure (Barret *et al*. 1985; Fikes 1990; Marrs 1993; Mosha 1993), but occasionally up to 21 days later (Stuart & Oehme 1982). It is characterized by an asymmetrical sensory-motor axonopathy as result of the inhibition of neuropathy target esterase (neurotoxic esterase [NTE]) (Aaron & Howland 1994; Fikes 1990; Marrs 1993), which is different from acetylcholinesterase (Stuart & Oehme 1982).

Only neurotoxic organophosphors bind irreversibly, by means of phosphorylation, to NTE. This process starts with hydrolysis of an ester or amide bond, leaving an ionized acidic group on the phosphorus atom (Meerdink 1989; Stuart & Oehme 1982). This process is time-dependent ("aging"), which will explain the prolonged time interval between exposure and the development of toxicity (Barret *et al*. 1985; Stuart & Oehme 1982). If acute exposure to an appropriate neurotoxic organophosphor results in more than 70 % inhibition of NTE, OPIDN usually follows (Stuart & Oehme 1982).

Not all organophosphors are neurotoxic, although they can also bind to NTE. As such, these non-neurotoxic organophosphors may paradoxically prevent neurotoxic effects by competing for NTE and do not undergo “aging” (Stuart & Oehme 1982).

The most severe clinical sign associated with OPIDN is paralysis of the limbs, while moderate cases show high-stepping gait and ataxia with the absence of pain (Barret *et al*. 1985; Fikes 1990; Marrs 1993; Stuart & Oehme 1982).

Triaryl phosphate is a neurotoxic compound, which induces OPIDN in cattle (Beck *et al*. 1977; Dollahite & Pierce 1969), while pigs showed neurotoxicity after dermal application of tri-o-tolyl phosphate (triorthocresyl phosphate) (Kruckenberg *et al*. 1973).

Histologically, the lesion involves a process known as Wallerian degeneration of the long axons of the peripheral nerves, as well as the ascending and descending tracts of the spinal cord (Stuart & Oehme 1982). There is a loss of the myelin sheath, proliferation of Schwann cells with macrophage accumulation (Barret *et al*. 1985; Dollahite & Pierce 1969). The thick myelinated fibres are more affected than the thin unmyelinated fibres (Stuart & Oehme 1982). The resultant axonal degeneration starts at the most distal part and proceeds towards the cell body (Beck *et al*. 1977). The proximal portion of the axon and nerve cell body remain unaffected (Barret *et al*. 1985).

Myelin sheath changes consist of hypertrophy of the agranular endoplasmic reticulum and distortion of neurofilaments (Barret *et al*. 1985; Stuart & Oehme 1982).

Organophosphors may also be mutagenic, carcinogenic and induce organ-specific toxicity in the heart, liver and kidneys (Marrs 1993). Teratogenicity can be caused by a reduction in nicotinamide dinucleotide (NAD) (Barret *et al*. 1985). Malformations such as cleft palate, hydronephrosis and hydroureter have been observed in rats poisoned by parathion (Barret *et al*. 1985). Fenchlorphos administered to pregnant rabbits induced multiple teratogenic manifestations in the off-spring, which included external hydrocephalus, microcephaly, aortic arch hypoplasia, cardiac septal defects, syndactylia and micromelia (Nafstad *et al*. 1983).

2.6.4. Pathophysiology of carbamates

Carbamates react with the serine group on acetylcholinesterase to yield a carbamylation of the serine hydroxyl group (Stevens & Breckenridge 2001). The carbamylation of acetylcholinesterase is reversible and the carbamylated complex will hydrolyze in time, (Haddad 1983(b); Stevens & Breckenridge 2001) usually within 48 hours (Aaron & Howland 1994). This is different from the organophosphors, which bind the esterases irreversibly and new enzyme is only resynthesized after 20 – 30 days (Desire & Saint-Andre 1987).

2.7. Clinical signs

The accumulated Ach excessively stimulates cholinergic receptors (muscarinic, nicotinic and in the central nervous system). Muscarinic effects such as salivation, miosis, lacrimation, urination, diarrhoea, bradycardia and bronchoconstriction with excessive bronchial secretions are dominant. Nicotinic effects manifest as tremors, muscle stiffness, weakness and paralysis (Desire & Saint-Andre 1987; Ecobichon 2001; Fikes 1990; Sommers 1992). Central nervous system effects include restlessness, confusion, ataxia, convulsions and cardiorespiratory depression (Aaron & Howland 1994; Mosha 1993). Mortalities are commonly attributed to respiratory failure (Aaron & Howland 1994; Fikes 1990; Matthew & Lawson 1979; Mosha 1993).

2.8. Necropsy findings

Post-mortem findings are mostly non-specific (e.g. congestion and cyanosis) and not consistent. Lesions include rupture of large bronchi, pulmonary oedema and emphysema and petechiation of some organs. Other lesions that have been reported are pancreatitis and enteritis in dogs and myopathy of the diaphragmatic and intercostal muscles in severe cases (Fikes 1990).

2.9. Diagnosis of organophosphor and carbamate poisoning

The history and clinical signs are important criteria in the diagnosis of suspected poisoning. Confirmation of toxicity can be obtained by analyzing the stomach or rumen contents for the presence of organophosphors or carbamates (Meerdink 1989). Determination of blood cholinesterase activity

is also a good indicator of organophosphor poisoning as it quantifies enzyme activity.

Acetylcholinesterase (true cholinesterase) is found primarily in nervous tissue and erythrocytes, while plasma cholinesterase (pseudocholinesterase or butyrylcholinesterase) is found in liver, other organs and plasma (Haddad 1983(a); Tecles & Ceron 2001; Tecles *et al*. 2000).

Variations in acetylcholinesterase activity between species are too great to establish a general reference range (Anderson *et al*. 1969) and are, therefore, a critical factor that influences interpretation of laboratory results (Blakley & Yole 2002). Thus, a database with normal values is needed for each species (Blakley & Yole 2002). Erythrocyte acetylcholinesterase inhibition is a useful tool to aid in the diagnosis of organophosphor poisoning in cattle and sheep, (Anderson *et al*. 1969) because 90 % or more of the total cholinesterase is found in the red blood cells (Tecles *et al*. 2000). Dogs and cats, on the other hand, have similar pseudocholinesterase and acetylcholinesterase activities (Tecles & Ceron 2001). In fish, significant differences exist regarding acetylcholinesterase and butyrylcholinesterase among different species, but if butyrylcholinesterase is present, it has a higher activity than acetylcholinesterase (Chuiko 2000). Cholinesterases in whole blood, plasma or brain are inhibited to a similar degree in goats, therefore, any depression of cholinesterase activity is a reliable index of exposure to organophosphors in this species (Abdelsalam 1987(a)).

Samples collected during the post mortem examination should, therefore, include stomach/rumen contents, whole blood (when possible), blood clots, brain, eyes (ocular fluid) and liver (Meerdink 1989).

Reduction of cholinesterase activity to less than 25 % of normal is seen in severe cases (Hansen 1995; Meerdink 1989; Mosha 1993), but a 50 % reduction is considered a significant inhibition (Meerdink 1989).

Although the determination of cholinesterase activity is the gold standard for confirmation of organophosphor poisoning it is not reliable to confirm exposure to carbamates, as the cholinesterases spontaneously reactivate and give false negative results.

2.10. Treatment of organophosphor and carbamate toxicoses

In companion animals it would appear that mild intoxication could be successfully treated, although the more severe cases usually die, despite intensive treatment.

The most important treatment is repeated parenteral administration of atropine at 0.1 - 0.2 mg/kg (Fikes 1990; Hansen 1995) in dogs and cats. The dose of atropine is 0.25 - 0.5 mg/kg in cattle and up to 1 mg/kg in sheep (Meerdink 1989). A total dose of 65 mg is recommended for the average horse (Meerdink 1989). The total dose of atropine in humans is only 2 mg intravenously (Matthew & Lawson 1979; Sommers 1992).

Atropine has no effect on nicotinic receptors and will not counteract muscle tremors, weakness or paralysis (Campbell & Chapman 2000; Ecobichon 2001). Diphenhydramine dosed at 1 - 4 mg/kg per os every 6 - 8 hours may be useful to counteract the nicotinic effects (Clemmons *et al*. 1984; Fikes 1990). Even a higher dose of 5 mg/kg diphenhydramine is also acceptable (Cordoba *et al*. 1983).

Enzyme reactivators are useful in the treatment of organophosphor poisoning, but not with carbamate poisoning, as the acetylcholinesterase inhibition in the latter is reversible and the enzyme will re-activate spontaneously in a short period of time irrespective of treatment (Fikes 1990; Haddad 1983(b); Meerdink 1989). The reactivators are used in organophosphor poisoning because of stronger and longer inhibition of acetylcholinesterase. They must, however, be administered within 24 hours before "aging" occurs, but preferably, within the first 12 - 18 hours (Meerdink 1989). Howland & Aaron (1994) suggested a maximum limit of up to 48 hours. Pralidoxime chloride (2-PAM) is administered at 10 - 15 mg/kg 2 - 3 times a day in dogs and cats (Hansen 1995). The reactivator competes for the phosphate moiety of the organophosphor compound, thereby releasing it from the acetylcholinesterase enzyme (Howland & Aaron 1994).

The clinician should also remove the poison to prevent further exposure and absorption. Further absorption from the stomach of dogs can be avoided by administering the emetics, apomorphine (0.04 mg/kg i/v or 0.08 mg/kg i/m or s/c (Bailey 1986)) or syrup of ipecac (1 - 2 ml/kg p.o. [not more than 15 ml in

total]) (Bailey 1986; Beasley & Dorman 1990). For cats the dosage of syrup of ipecac is 3.3 ml/kg p.o. (Beasley & Dorman 1990; Dorman 1995). Gastric lavage in small animals or rumenotomy in large animals can also be considered.

Adsorbents e.g. activated charcoal 1 - 4 g/kg p.o. (Beasley & Dorman 1990; Buck & Bratich 1986; Dorman 1995) are very effective in binding ingested pesticides. A cathartic must be used at the same time, because activated charcoal becomes stationary in the gastro-intestinal tract and slowly releases the adsorbed toxin. The cathartic promotes passage of the activated charcoal and elimination of the adsorbed toxin via the faeces (Buck & Bratich 1986).

Additional supportive treatment must be given, which could include light anaesthesia or deep sedation and fluid therapy until the dog has eliminated the poison.

2.11. Cyclodextrins

2.11.1. History

Cyclodextrin chemistry dates back to 1903 - 1911, when Schardinger discovered that thermophilic bacteria were able to dissolve starch to form crystalline polysaccharides (“dextrins”) (Clarke *et al*. 1988). The bacterium, which produced the best dextrins, was *Bacillus macerans*. The next major contribution came from Freudenberg and Jacobi, who developed a method to obtain pure alpha and beta cyclodextrins. They proposed the theory of a ring structure in 1936 and managed to determine the chemical structures of the

dextrins. Freudenberg used the cryoscopic method to determine the molecular weights, which gave incorrect values for the number of D-glucosyl residues of alpha and beta dextrin rings (Clarke *et al*. 1988). French obtained the molecular weights by X-ray diffraction combined with crystal density measurement. Freudenberg later concurred with French's results (Clarke *et al*. 1988). From these earlier natural cyclodextrins, several synthetic cyclodextrins were developed to complex certain drugs and toxins.

2.11.2. Structure

Cyclodextrins (Figure 5), capable of binding toxins of low molecular weight, are toroidal ("doughnut-shaped") oligosaccharides produced from starch by the bacterium *Bacillus macerans* (Clarke *et al*. 1988; Desire & Saint-Andre 1987; Martin Del Valle 2004) and contain 6, 7 or 8 α-D-glucopyranosyl residues referred to as α, β and γ-cyclodextrins, respectively (Clarke *et al*. 1988; Loftsson & Masson 2001; Martin Del Valle 2004). The space within the cyclodextrin (CD) cavity increases with the number of D-glucopyranosyl residues (Clarke *et al*. 1988).

Cyclodextrins have a hydrophilic exterior, but a hydrophobic interior cavity. Due to its cyclic structure, cyclodextrins have the ability to form inclusion complexes with many lipophilic molecules (Loftsson & Masson 2001). The extent of the complex formation depends on the polarity of the guest molecule.

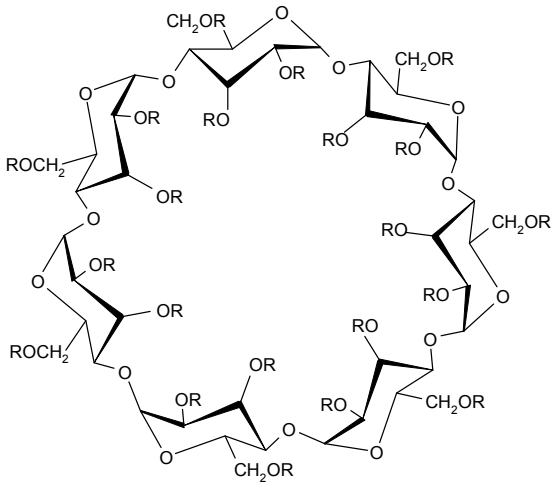

Figure 5: Hydroxypropyl-β-cyclodextrin

Strongly hydrophilic and ionised groups are not (or weakly) complexable (Fromming & Szejtli 1994). Cyclodextrins may host a great variety of molecules, of molecular mass less than 250 (Walker 1993) or having the size of one or two benzene rings, or even larger ones with a side chain of comparable size (Fromming & Szejtli 1994). Thus, the size and polarity of the guest molecule will influence the ability to complex with cyclodextrin. The complex renders lipid soluble molecules relatively more water-soluble and aids renal excretion. Molecules that are hydrophobic, or have a fatty acid side chain, will partition inside the cavity of the cyclodextrins forming a molecular complex, changing the physical and biochemical properties of the "guest" molecule (Stewart *et al*. 1998).

2.11.3. Energy dynamics during complex formation

For a complex to form, there must be a favourable nett energetic driving force that pulls the guest into the cyclodextrin (Martin Del Valle 2004). The four energetic interactions that form the inclusion complex are as follows:

- Water molecules are expelled from the apolar cyclodextrin cavity.

- Hydrogen bonds increase, due to the displaced water returning to the larger pool.
- A decrease in the repulsive interactions between the lipophilic guest and the aqueous environment.
- An increase in the hydrophobic attractions, as the guest binds itself into the apolar cyclodextrin cavity (Fromming & Szejtli 1994; Martin del Valle 2004).

2.11.4. Types of cyclodextrins

(a) Natural cyclodextrins

The natural cyclodextrins, namely α, β and γ-cyclodextrins had some disadvantages. The cost of γ-cyclodextrin makes its use economically unfavourable. β-Cyclodextrin has two problems, namely limited water-solubility and nephrotoxicity (Rajewski & Stella 1996).

(b) Modified cyclodextrins

By modifying the natural cyclodextrins, various new compounds have been synthesized to circumvent the disadvantages of poor water-solubility and toxicity (Rajewski & Stella 1996). Hydroxypropyl-β-cyclodextrin (Fig 2.5) is highly water-soluble and safe, even at high doses (Frijlink *et al*. 1991; Fromming & Szejtli 1994).

2.11.5. Pharmacokinetics of cyclodextrins

(a) Oral administration

Cyclodextrins are slowly absorbed, as they need a concentration gradient and a passive transport system (Walker 1993). Cyclodextrins are carriers of molecules, which enhance the absorption of the latter from the intestinal tract. They transport guest molecules to the lipophilic cell membrane. Dissolution of the cyclodextrin-guest complex, leads to rapid absorption of the guest molecule, but limited amounts of the carrier are absorbed (Fromming & Szejtli 1994; Irie & Uekama 1997). Cyclodextrins are mainly metabolized in the colon by microflora. The primary metabolites, namely acyclic maltodextrins, maltose and glucose, are further metabolized or absorbed.

(b) Intravenous administration

After intravenous injection in rats, β-cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) rapidly distribute in extracellular fluids and are eliminated by glomerular filtration (Frijlink *et al*. 1991; Irie & Uekama 1997). The pharmacokinetics of HP-β-CD was linear up to a dose of about 200 mg/kg, whereas the elimination rate of β-CD decreased when the dose exceeded 100 mg/kg (Frijlink *et al*. 1991). The steady-state volume of distribution of β-CD and HP-β-CD in rats, rabbits, dogs and humans correspond well with the extracellular volume of each species, thus no deep compartments or storage pools are involved (Irie & Uekama 1997). The total plasma clearance for HP-β-CD in all species tested, are similar to that of inulin, a polysaccharide known to be rapidly distributed in the extracellular fluid and excreted at the rate of glomerular filtration (Irie & Uekama 1997).

For both HP-β-CD and β-CD over 90 % of the dose is excreted unchanged in the urine within 24 hours after administration (Frijlink *et al*. 1991). The renal clearance of HP-β-CD in humans is 110 - 130 ml/min, which is independent of the dose administered and nearly equivalent to the glomerular filtration rate of 125 ml/min (Irie & Uekama 1997).

(c) Transmucosal administration

2,6-Dimethyl-β-cyclodextrin (DM-β-CD) is useful as a transmucosal nasal absorption enhancer of therapeutic peptides and proteins (Irie & Uekama 1997). Intratracheal administration of β-CD, DM-β-CD and HP-β-CD resulted in much higher absorption rates of these CD's compared to other mucosal routes such as oral, rectal and nasal administration (Irie & Uekama 1997).

(d) Dermal administration

The stratum corneum acts as a barrier to the penetration of HP-β-CD through skin (Irie & Uekama 1997). The absorption of cyclodextrins through the skin can be improved by combining them with absorption-promoting agents such as polyvinylpyrrolidone (Rajewski & Stella 1996).

2.11.6. Toxicity of cyclodextrins

(a) Oral administration

Large oral doses of HP-β-CD can bind lipophilic toxins and carcinogens and enhance their dissolution and absorption from the intestinal tract (Gerloczy *et al*. 1994; Horsky & Pitha 1996). Therefore, if cyclodextrins are used orally, it

must be in combination with another beneficial drug, which will complex the cyclodextrin.

A 52-week chronic toxicity study was performed in rats and dogs by dietary administration of β-CD. Ingestion rates of 654, 1313 and 2655 mg/kg/day for male rats and 864, 1743 and 3614 mg/kg/day for female rats were determined. The doses for the dogs were 229, 456 and 1831 mg/kg/day for males and 224, 476 and 1967 mg/kg/day for females. No effect on body weight gain, food consumption, ophthalmoscopic findings, haematological parameters, mortality rate, organ weights or macroscopic pathology was noticed (Bellringer *et al*. 1995).

Evaluation of the potential of β-CD to induce teratogenicity was studied in rats at doses of up to 5000 mg/kg administered during Days 7 -16 of pregnancy. No evidence of teratogenicity or foetotoxicity was observed in any of the studies (Walker 1993).

(b) Parenteral administration

α- and β-Cyclodextrins are nephrotoxic, due to the processes of glomerular filtration and reabsorption by renal proximal convoluted tubular epithelial cells. Cyclodextrins accumulate in the tubular cytoplasm, forming crystals, resulting in an increase in lysosomal activity and necrosis (Irie & Uekama 1997; Rajewski *et al*. 1995). Intramuscular and subcutaneous administration can lead to necrosis of tissue surrounding the injection site. Cyclodextrins are

potentially haemolytic and polysulphated-β-cyclodextrin has been shown to prolong thrombin clotting times (Rajewski *et al*. 1995).

Several acute toxicity studies of cyclodextrins have been conducted in mice, rats and dogs (Bellringer *et al*. 1995; Fromming & Szejtli 1994; Walker 1993). Intravenous LD_{50} of β-cyclodextrin in rats was calculated as 788 mg/kg (Fromming & Szejtli 1994; Walker 1993).

(c) Toxicity of hydroxypropyl-β-cyclodextrin

Intraperitoneal administration of HP-β-CD in mice did not induce any toxic effect over a 30 day observation period (Rajewski *et al*. 1995). Hydroxypropyl-β-cyclodextrin has a very low toxicity after parenteral administration and no adverse effects have been observed in humans (Carpenter *et al*. 1995). However, occasionally they may induce pulmonary oedema in dogs and distress in rabbits (Carpenter *et al*. 1995; Irie & Uekama 1997). The toxicity of HP-β-CD has been assessed in rats and monkeys in acute, subacute (14 days) and subchronic (90 days) studies, with no visible effects (Fromming & Szejtli 1994). Parenteral doses as high as 10000 mg/kg administered to monkeys were not lethal. In mice intraperitoneal administration up to 10000 mg/kg and intravenous administration up to 2000 mg/kg did not cause death (Fromming & Szejtli 1994). The mild renal changes observed at high HP-β-CD doses are reversible (Irie & Uekama 1997). Oral administration of HP-β-CD in human trials, showed no toxicity if the daily dose is less than 16 grams. Hydroxypropyl-β-cyclodextrin has no mutagenic,

carcinogenic, teratogenic or embryotoxic potential, neither any adverse effects on fertility and postnatal development (Irie & Uekama 1997).

2.11.7. Clinical uses of cyclodextrins

Cyclodextrins enhance topical drug delivery by increasing the drug availability at the barrier surface (Loftsson & Masson 2001). Due to their size and hydrophilicity, only insignificant amounts of cyclodextrins or drug-cyclodextrin complexes can penetrate lipophilic barriers, such as skin (Loftsson & Masson 2001). Cyclodextrins act as true carriers by keeping hydrophobic drugs in solution and delivering them to the surface of a biological membrane. They act as penetration enhancers by increasing drug availability at the surface of the biological barrier (Martin Del Valle 2004). Cyclodextrins are also utilized for the stabilisation of light - or oxygen sensitive drugs; modification of chemical reactivity of guest molecules; fixation of very volatile substances; improvement of solubility of substances; protection against degradation of substances by micro-organisms as well as to mask ill smell, taste or colour of substances (Martin Del Valle 2004).

In the pharmaceutical industry, cyclodextrins are used to increase the absorption and bioavailability of drugs. Numerous examples exist in this regard. Morphine, a potent opioid analgesic, was evaluated in rectal suppositories in combination with α-cyclodextrin and xanthan gum. The latter prevented rectal irritation by α-cyclodextrin. Morphine release from the suppository was enhanced and rectal absorption was increased (Uekama *etal*. 1995). The solubility and oral bioavailability of miconazole, a broad-

spectrum antifungal agent, increased when it was combined with HP-β-CD (Tenjarla *et al*. 1998). A formulation containing HP-β-CD buffered at pH 6.5 - 7 is suitable for dissolution, stabilization and administration of diclofenac eye drops (Reer *et al*. 1994).

2.12. Justification for the research approach

2.12.1. Reason for the survey

Worldwide, several outbreaks of aldicarb poisoning have been reported (De Bosschere *et al*. 1999; Frazier *et al*. 1999; Grendon *et al*. 1994; Kerr *et al*. 1991). Poisoning of dogs and cats with aldicarb occurs in South Africa and numerous incidences of intentional poisoning have also been reported in the media. Therefore, it was decided to conduct a retrospective survey in Gauteng Province to better gauge the incidence of aldicarb poisoning in the country.

2.12.2. Suitability of hydroxypropyl-β-cyclodextrin as a binding agent for aldicarb

The following factors regarding HP-β-CD, can be considered as favourable to be used as a binding agent for aldicarb:

- The molecular weight of aldicarb is 190.3 dalton (Tomlin 1994) and therefore small enough to fit into the HP-β-CD cavity, as most CD's can bind molecules of molecular weight of 250 or less (Walker 1993)
- Aldicarb is moderately lipid-soluble (Tomlin 1994)

- Aldicarb is non-ionized and such molecules can be complexed (Fromming & Szejtli 1994)
- Safety profile of parenterally administered HP-β-CD.

CHAPTER 3

ALDICARB POISONING OF DOGS AND CATS IN GAUTENG DURING 2003

Article published in the
Journal of the South African Veterinary Association (2004) 75(4): 177-181

Article — Artikel

Aldicarb poisoning of dogs and cats in Gauteng during 2003

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3.1. ABSTRACT

A survey of aldicarb poisoning in companion animals was conducted by posting questionnaires to all private practitioners in Gauteng Province, South Africa. The survey was designed to determine the percentage of aldicarb cases seen, treatment regimen, clinical signs observed, proposals for preventative actions and more effective treatments. Other questions included duration of treatment, survival rate, cost to clients, *post mortem* findings and reasons for poisonings.

Key words: aldicarb, cats, dogs, poisoning, treatment.

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Received: September 2004. Accepted: October 2004.

3.2. INTRODUCTION

Pesticides are frequently and widely used to protect crops from various pests. Annually, 500 000 tonnes are produced in the United States of America, representing over 900 compounds, which are formulated into 25 000 registered products[9].

Both the organophosphors and carbamates are important and widely available agricultural pesticides in South Africa. Following exposure in animals the carbamates react with the serine group of acetylcholinesterase to yield a carbamylation of the serine hydroxyl group[20]. This carbamylation is reversible and the carbamylated complex hydrolyses in time[13,20]. On the other hand, the organophosphor compounds phosphorylate acetylcholinesterase, but unlike the carbamates, the inhibition becomes irreversible after 24–48 hours[12].

The inhibition of acetylcholinesterase promotes the accumulation of acetylcholine, which initially excites and then paralyses transmission at muscarinic cholinergic synapses in the central nervous system, parasympathetic nerve endings and a few adrenergic nerve endings, such as sweat and salivary glands[12]. The nicotinic cholinergic receptors at somatic nerve endings and the ganglionic synapses of autonomic ganglia are also affected[12].

Clinically, muscarinic-induced effects include hypersalivation, lacrimation, urination, diarrhoea, bradycardia, bronchoconstriction with excess bronchial

secretions and miosis[3,8,9]. Nicotinic effects manifest as tremors, muscle stiffness, weakness and paralysis[3,8,9]. The muscular hypertonia, tremors and convulsions can lead to exertional rhabdomyolysis[16]. Mortalities are commonly attributed to respiratory failure[8,9].

Aldicarb ('Temik', Bayer CropScience), an oxime carbamate insecticide and nematocide, is registered in terms of the Fertilisers, Farm Feeds, Agricultural and Stock Remedies Act, 1947 (Act 36 of 1947). Aldicarb is one of the most toxic pesticides, with a rat oral LD_{50} of 0.93 mg/kg[21]. Worldwide, several incidents of aldicarb poisoning have been reported6,[10,11,15] and malicious poisoning of dogs and cats with aldicarb occurs frequently in South Africa. The Division of Toxicology, Onderstepoort Veterinary Institute (OVI) confirmed 72 cases in 1998, 67 cases in 1999 and 72 cases in 2000; this increased to 115 in 2001 and 114 in 2002. The majority occurred in dogs and cats, but sporadic occurrences in cattle, birds, monkeys and antelope were also reported. In 2003, 97 cases of aldicarb poisoning were confirmed which included 32 dogs and 6 cats in the province of Gauteng (J P J Joubert, ARC-Onderstepoort Veterinary Institute, pers. comm., 2004). However, these official figures are not considered to be representative of the actual number of aldicarb poisonings, as numerous incidences of malicious poisoning have been reported in the media.

In order to better gauge the incidence of aldicarb poisoning in the country, a retrospective survey was conducted in Gauteng Province, South Africa.

3.3. MATERIALS AND METHODS

A questionnaire, designed to gain information on the incidence of aldicarb poisoning in dogs and cats, was compiled and sent to all veterinary facilities in Gauteng registered with the South African Veterinary Council. Veterinary practitioners were requested to provide an estimate of their total caseload and the related incidence of organophosphor and/or carbamate poisonings with specific focus on aldicarb poisonings. They were also asked to indicate how many suspected aldicarb cases were confirmed after submitting specimens to the Onderstepoort Veterinary Institute or other laboratories for chemical analysis.

The veterinarians were also requested to provide information on the frequency of clinical signs observed and their treatment regimen by ticking boxes marked *always, often, sometimes* or *never.* Clinical signs listed in the questionnaire included tremors, salivation, emesis, miosis, diarrhoea, urination, dyspnoea, bradycardia and seizures. They were asked to point out any other clinical signs not listed. The treatment options included administration of atropine, activated charcoal, fluid therapy, enzyme reactivators, diphenhydramine, oxygen therapy, induction of emesis or gastric lavage and no treatment, *i.e.* when euthanasia is performed. The veterinary practitioners were also asked to propose any other effective treatment.

Other specific questions focused on average duration of treatment, cost of

treatment, survival rate, *post mortem* lesions, reasons for poisoning, suggested preventative measures, perceived seasonal occurrence and whether there was an increase in the incidence of poisonings.

All the data from the returned questionnaires were captured in Microsoft Access. The geographical maps were compiled using ArcInfo (ESRI, New York). Practice locations were determined by geocoding their street addresses through the web service AfriGIS (www.afrigis.co.za).
Density determination was done by the Kernel method (bandwidth: 5 km, cell size:1 km^2) using the Spatial Analyst extension to ArcInfo.

3.4. RESULTS

Of the 315 questionnaires posted, 97 were returned and 12 were not delivered. However, 14 of the completed questionnaires were submitted from outside the borders of Gauteng and were excluded. Thus, 83 out of 289 questionnaires were included, furnishing a response of 28.7 %.
Only one third (34 %) of respondents indicated the total number of all clinical cases presented to their clinics during 2003. The percentage of suspected aldicarb cases as a proportion of all cases ranged from 0.05–2.6 % for dogs and 0.09–3.33 % for cats. One clinic estimated an incidence of 6 % (3 aldicarb cases out of 50 feline patients in total), which is much higher than all the other veterinary practices (Figure 6-Figure 9). A quarter of respondents (26.5 %) sometimes submitted samples for laboratory confirmation of an aldicarb diagnosis.

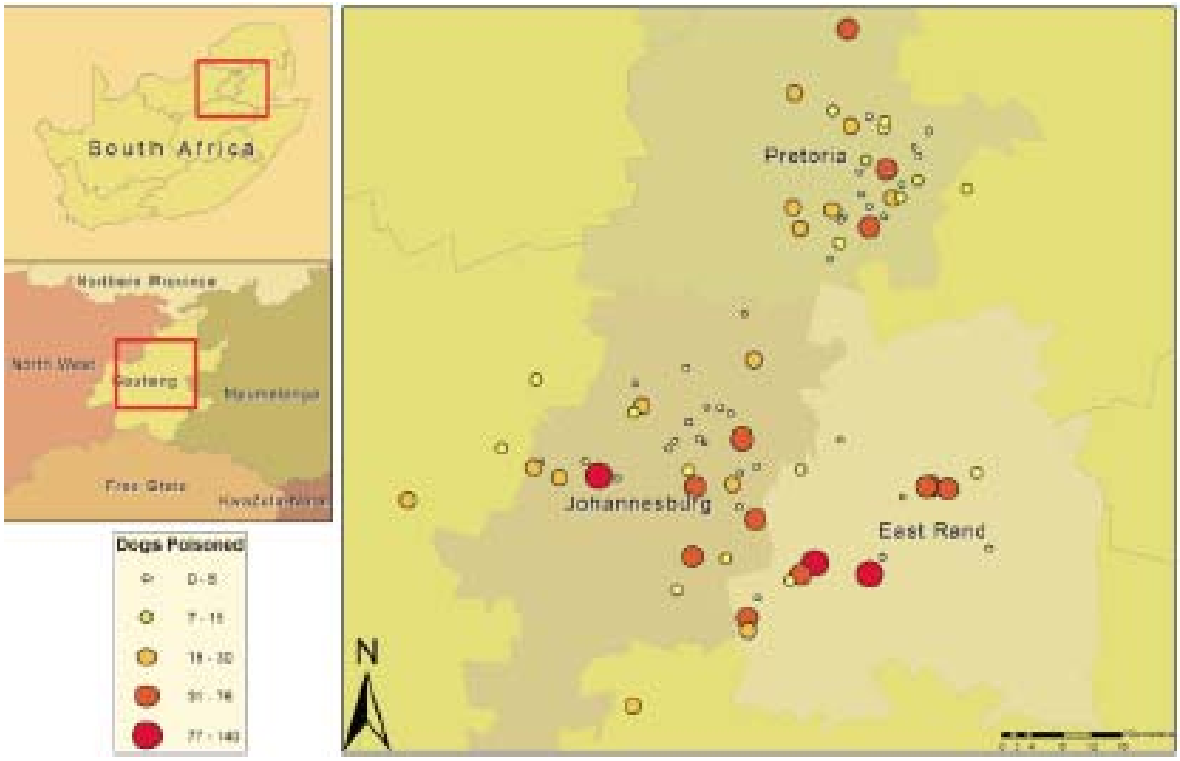

Figure 6: **Estimated number of aldicarb cases in dogs**

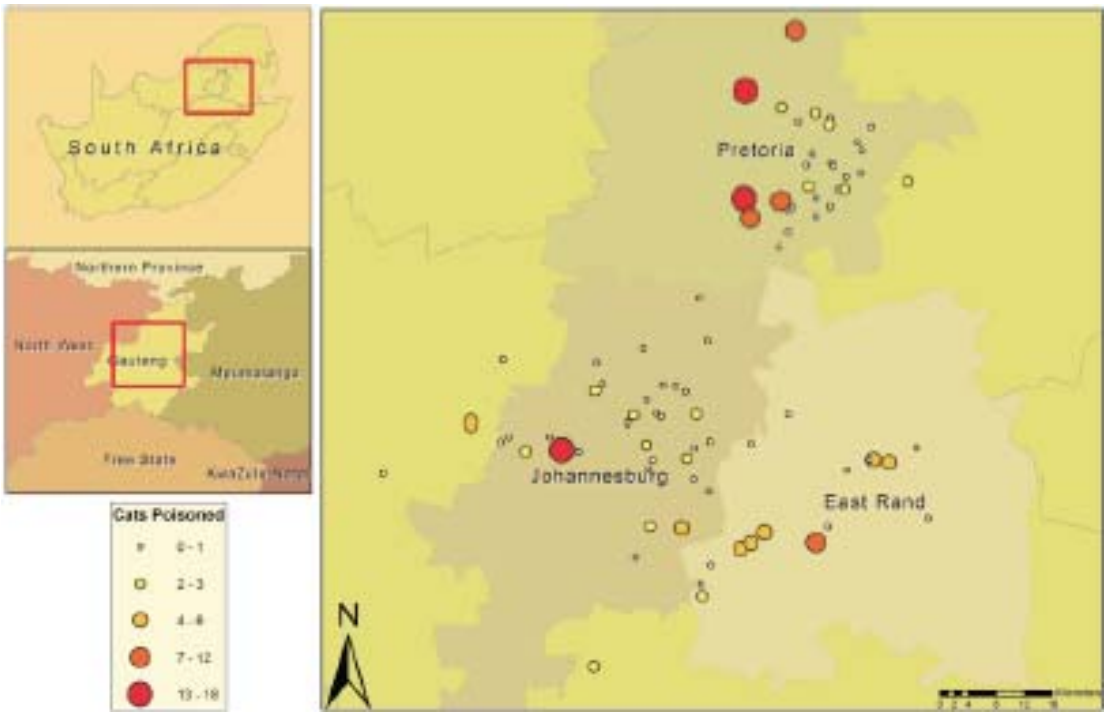

Figure 7: **Estimated number of aldicarb cases in cats**

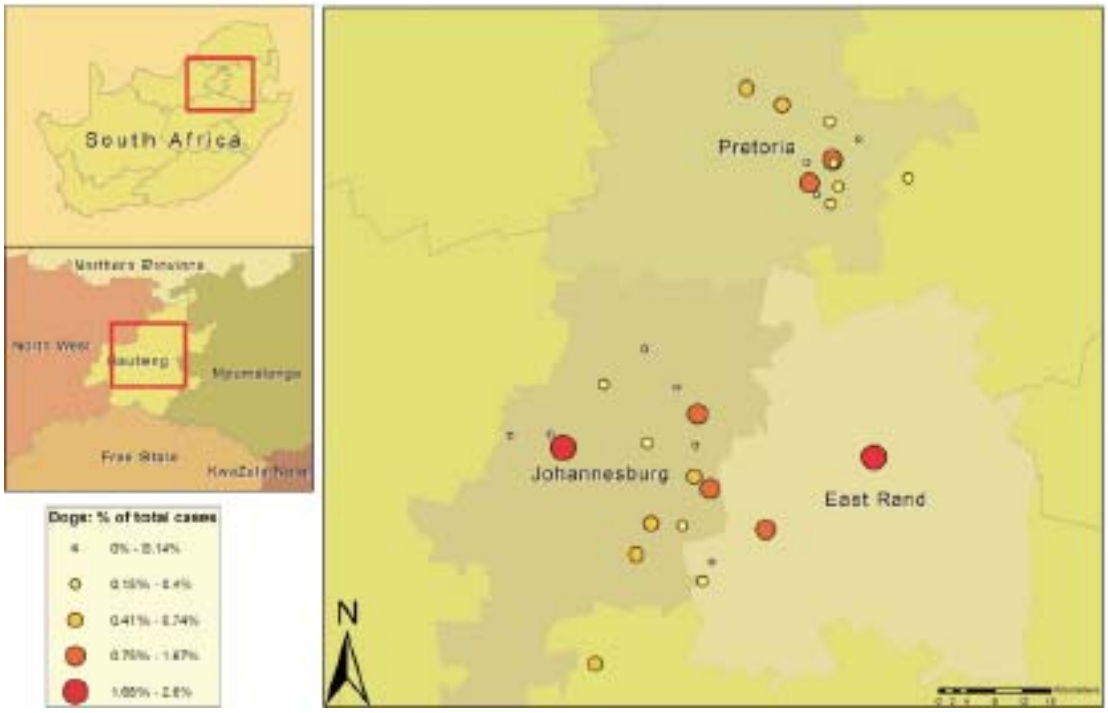

Figure 8: **Alicarb poisonings as percentage of total caseload in dogs**

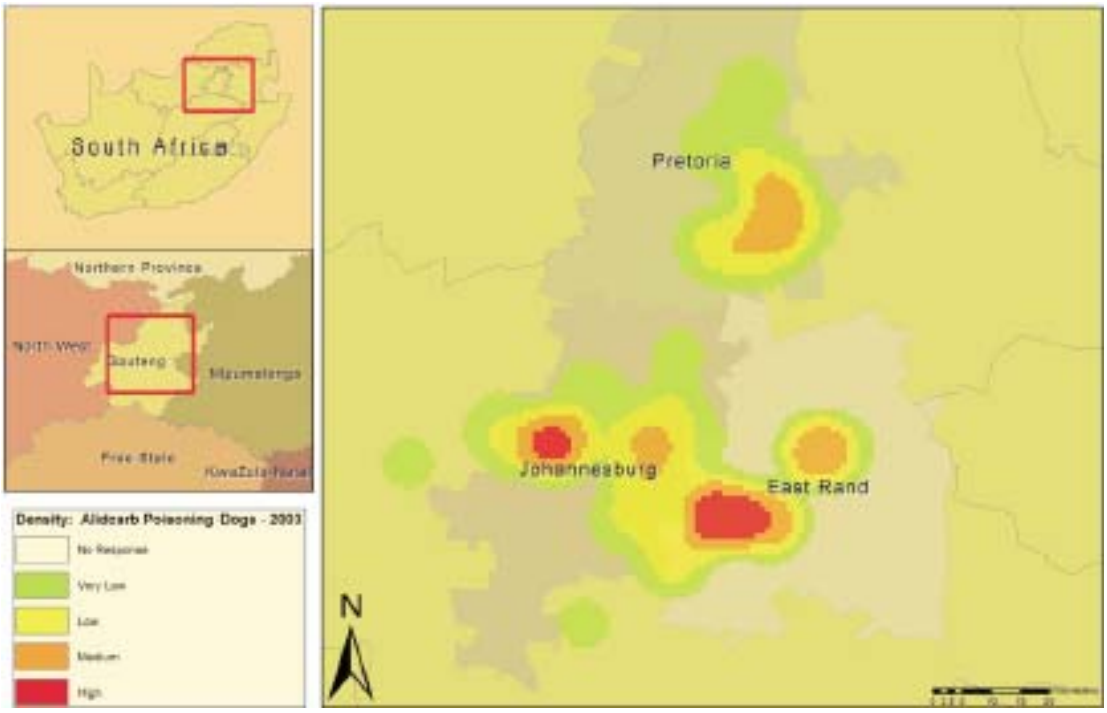

Figure 9: **Density distribution of aldicarb poisonings in dogs**

Frequency of clinical signs observed as reported by the veterinary practitioners are depicted in Figure 10.

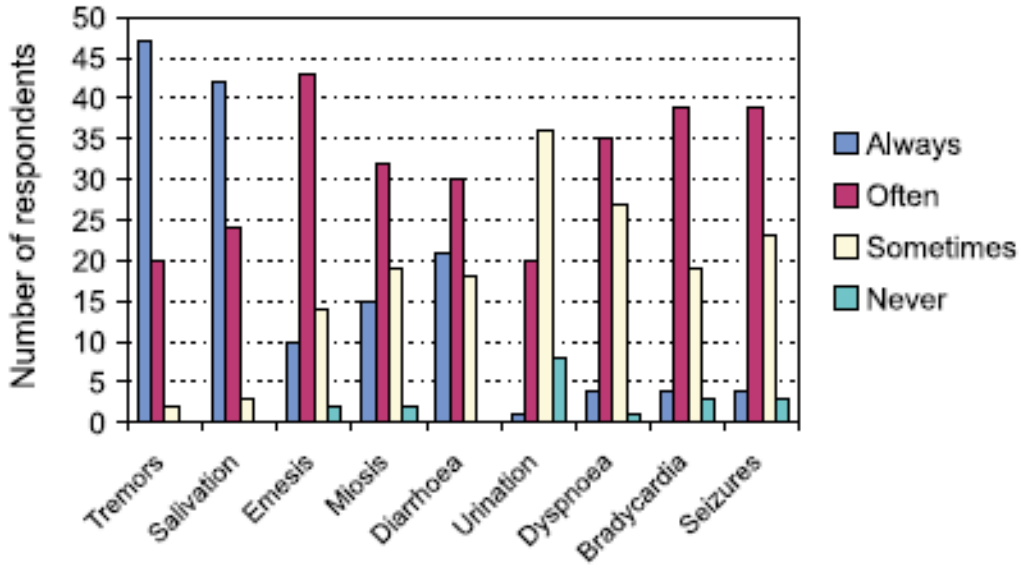

Figure 10: Clinical signs observed by veterinarians

The majority of veterinarians always observed tremors and salivation. Emesis, seizures, bradycardia, dyspnoea and miosis were also often seen. Although diarrhoea was frequently observed, excessive urination was only occasionally recorded. In addition, paresis or paralysis was also noted.

Most animals suffering from suspected poisoning were treated with atropine. Intravenous fluids and electrolyte therapy as well as activated charcoal were also used frequently. The enzyme reactivators and diphenhydramine were seldom administered (Figure 11).

Other treatments include liver supportive therapy (thioctic acid), vitamin B complex, benzodiazepine sedatives (diazepam) and intravenous anaesthetic agents (pentobarbitone). The clinicians also suggested the use of the bronchodilator, aminophylline and intravenous colloids and loop diuretics (furosemide) to alleviate pulmonary oedema. Non-specific treatments such as anti-inflammatory agents and analgesics were also sometimes used.

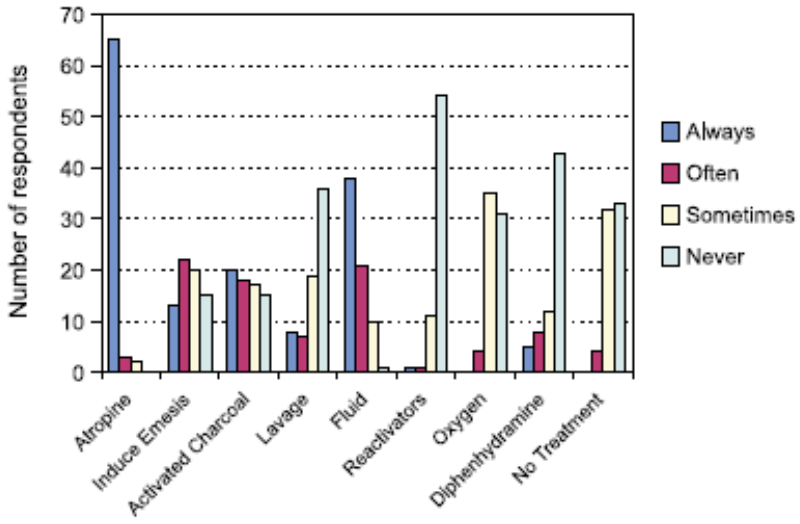

Figure 11: **Treatment regimen of veterinary practitioners**

Two thirds of respondents estimated a 50–75 % survival rate following treatment, which could extend from 1–7 days, with probability of survival increasing in animals when treatment is initiated as soon as possible. The average cost of treatment varied from ZAR500.00 to 1500.00 and was related to the duration of hospitalization and range of treatments.

Pet owners were advised of the following preventative measures: keep dogs inside at night or in back yard; feed dogs at night to thwart ingestion of baits; obedience training for dogs to prevent food acceptance from strangers; check on the animals and for unfamiliar food in the garden early in the morning and do not leave vehicles and valuable items outside. The veterinarians are of the opinion that the incidence of poisoning should not be published in the local media, that an aversive substance could be included in the aldicarb granular formulation, that the sale of aldicarb to unauthorized persons should be prohibited and that the law should be enforced.

No seasonal occurrence was noticed with 95 % of respondents indicating that poisoning occurred throughout the year. However, there was an impression that cases of malicious poisoning increased during holiday periods.

Few respondents conducted *post mortem* examinations, but when it was performed the following macroscopic findings were noted: generalised congestion, petechial haemorrhages, lung oedema, enteritis and aldicarb granules in stomach.

Thirty-three respondents thought that there was an increase in the number of aldicarb cases, but 34 felt there was no increase during 2003. Sixteen respondents were reluctant to venture an opinion (Figure 12).

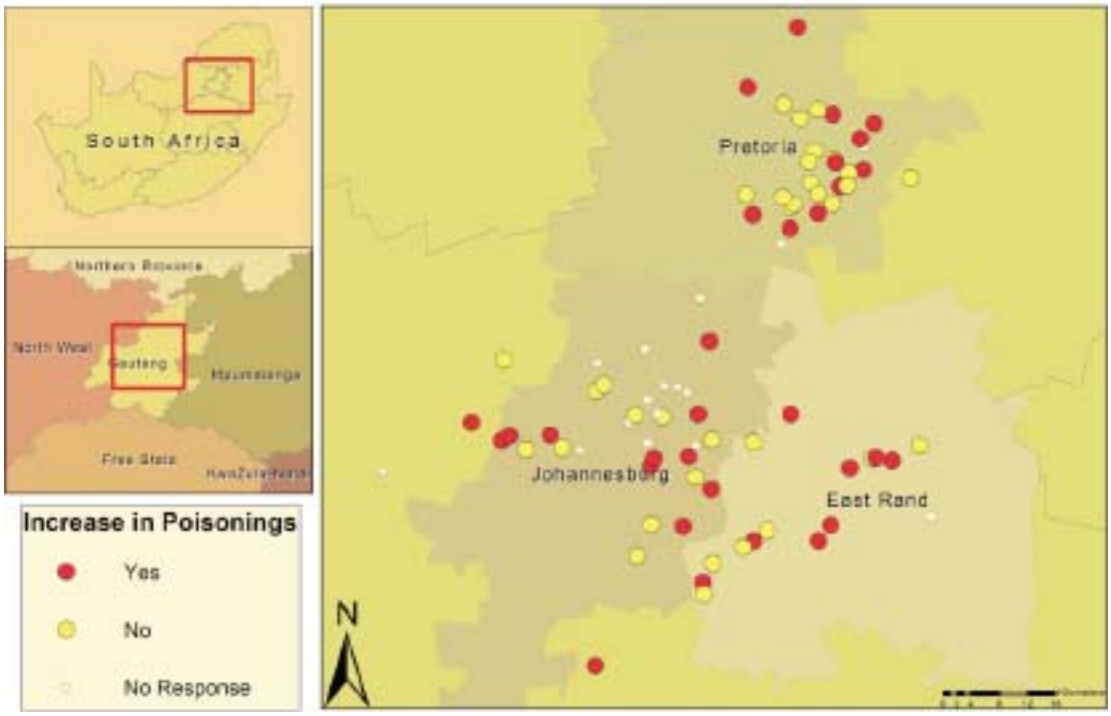

Figure 12: **Estimated incrase in aldicarb cases reported by veterinarians**

The majority of veterinarians (80 %) indicated that they thought criminal intent was the main reason why animals were poisoned. Most veterinarians indicated that animals were presented within 1–2 hours after owners noticing symptoms, but delays can occur due to transport problems.

3.5. DISCUSSION

The survey was conducted in Gauteng, the most populous and affluent province of South Africa, and although it may not accurately reflect the situation in the rest of the country, it is a good indicator of the occurrence of aldicarb poisoning in pets.

Veterinarians in the greater Pretoria area reported that most cases in dogs occur in the eastern suburbs and parts of Centurion. The majority of cases observed in dogs in the greater Johannesburg area occur in the central and eastern areas and on the East Rand. It appears that Roodepoort, Benoni and Boksburg are experiencing a high incidence of malicious poisoning (Figure 6,Figure 8).

It is, however, important to note that the density distribution (Figure 9) only reflects the density of cases as reported by the respondents. Areas with poor response rates could therefore still have had a high density of cases, although it could not be accurately reflected on the map, due to the lack of data.

The incidence of poisoning in cats follows a different pattern and they are probably often unintentionally poisoned when bait is placed out (Figure 7).

However, the exact prevalence of aldicarb poisonings in dogs and cats will

most likely never be fully known, as not all cases are presented to veterinarians.

Chemical analysis also adds substantially to the cost to the client, therefore only some veterinarians occasionally submit samples to confirm their diagnosis. In addition, malicious poisonings of dogs and cats are also not reported to the police.

The cost of treatment is expensive with no guarantee of a successful outcome. Current treatments seem to be reasonably successful, but hospitalisation with intensive therapy is often necessary. Some owners might request euthanasia for financial considerations or to prevent further suffering.

As can be expected, the majority of veterinarians administer atropine. When poisoning induced by an acetylcholinesterase inhibitor is suspected, the parenteral administration of atropine at 0.1–0.2 mg/kg[9,14] in dogs and cats is indicated. Atropine, a muscarinic antagonist, has no effect on nicotinic receptors and will not counteract muscle tremors, weakness or paralysis[3,9]. Diphenhydramine dosed at 1–5 mg/kg *per os* every 6–8 hours may be useful to reverse the nicotinic effects[4,5,9]. Gauteng veterinarians only seldom use diphenhydramine.

The use of enzyme reactivators in carbamate poisoning is controversial as the acetylcholinesterase inhibition is reversible and the enzyme reactivates

spontaneously[9,13,17]. On the other hand, the enzyme reactivators are useful in organophosphor poisoning, but only if administered before 'aging' occurs, thus within 24 hours or preferably within the first 12–18 hours[17].

Drugs such as phenothiazine tranquilizers, benzodiazepine sedatives, and aminoglycoside, clindamycin and lincomycin antibiotics[3,9,17] are contraindicated as they have neuromuscular blocking properties or compete for esterase enzymes. Central nervous system depressants such as the barbiturates and morphine must also be avoided, due to their respiratory depression tendencies[9]. Aminophylline should preferably not be administered as it has analeptic properties, which could exacerbate central nervous system stimulation[19].

Decontamination procedures such as induction of emesis and/or gastric lavage, in conjunction with adsorbents such as activated charcoal, can be effective to limit gastro-intestinal absorption. Activated charcoal, 1–4 g/kg[1,2,7] dosed orally, is an inert and safe compound. A saline cathartic should be administered after half-an-hour as activated charcoal becomes stationary in the gastro-intestinal tract and slowly releases the adsorbed toxin[2].

Pets that have died from suspected poisoning should be considered hazardous and every attempt should be made to ensure the correct disposal of the carcass. The remains should not be collected by municipal services and disposed of on municipal dumping sites, as there are always carrion-eating

birds. Incineration is probably the best method of disposal of a poisoned animal.

To prevent intoxication, the manufacturer of 'Temik' has included several safety measures in this aldicarb formulation (P Fourie & R Jones, Bayer Crop-Science, pers. comm., 2004). The 'Temik' granules, which contain 15%aldicarb, are sieved to remove dust and lumps, thus limiting the inhalation risk. The granules are coated with a flow agent to assist with agricultural application and to decrease skin contact, thus preventing percutaneous absorption. 'Temik' granules also contain an outer layer of 'Bitrex' (denatonium benzoate), a strong, bitter agent to discourage ingestion[18].

The product is registered under Act 36 of 1947, as a restricted use pesticide. Under this Act, only a qualified dealer, registered as an Aldicarb Pest Control Officer, may sell products containing aldicarb. This dealer must have completed suitable and thorough training. Farmers may only purchase 'Temik' after completing similar training courses and examinations. All storage facilities, including farm stores, must be inspected and comply with set standards. In addition, each 'Temik' container is identified by a unique serial number allowing the details of supply from site of manufacture to final user to be traced.

Despite all the legal requirements and control measures, some 'Temik' is still obtained illegally and used for unlawful purposes. Criminal intent is the most

important reason provided by the majority of veterinary practitioners for cases of aldicarb poisoning.

The illegal possession of aldicarb will continue to be a major cause of concern, but the economic benefits gained by the registered usage of 'Temik' on potatoes, citrus and other crops, are such that 'Temik' is recognised as essential for agricultural production. The solution is thus, not to withdraw an excellent pesticide from the market, but to keep it away from unauthorised persons by enforcing the law.

3.6. ACKNOWLEDGEMENTS

The financial contributions received from Bayer CropScience and the Faculty of Veterinary Science are gratefully acknowledged. The authors thank all the Gauteng private veterinarians who completedandreturnedthe questionnaire.

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CHAPTER 4

EVALUATION OF HYDROXYPROPYL-β-CYCLODEXTRIN IN THE TREATMENT OF ALDICARB POISONING IN RATS

Article published in the
Journal of the South African Veterinary Association (2004) 75(4): 182-185

Short communication — Kort berig

Evaluation of hydroxypropyl-_-cyclodextrin in the treatment of aldicarb poisoning in rats

R S Verster [a] and C J Botha

4.1. ABSTRACT

Cyclodextrins are ring-shaped oligosaccharides with a hydrophilic exterior and a hydrophobic interior. The interior cavity is capable of complexing fat-soluble molecules small enough to fit inside. Sprague-Dawley rats were used to evaluate the efficacy of hydroxypropyl-_-cyclodextrin as treatment of aldicarb poisoning in rats. Survival times in the majority of rats dosed with aldicarb and receiving intravenous cyclodextrin were longer compared with the control rats only dosed with aldicarb *per os.*

Key words: aldicarb, cyclodextrin, poisoning, rats.

Verster R S, Botha C J **Evaluation of hydroxypropyl-_-cyclodextrin in the treatment of aldicarb poisoning in rats**. Journal of the South African Veterinary Association (2004) 75(4): 182–185 (En.). Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

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Received: June 2004. Accepted: September 2004.

4.2. INTRODUCTION

Organophosphor and carbamate compounds are some of the most widely used pesticides, making it inevitable that accidental or intentional exposure of animals and humans will occur. Worldwide, several incidences of aldicarb poisoning have been reported[6,10,13,14] and malicious poisoning of dogs with aldicarb occurs frequently in South Africa. Data of confirmed cases of aldicarb mortalities obtained from the Toxicology Division, Onderstepoort Veterinary Institute, indicate the following: 72 cases were confirmed during 1998, 67 cases in 1999 and 72 cases were recorded in 2000; this increased to 115 in 2001 and 114 in 2002 and decreased somewhat to 97 in 2003 (J P J Joubert, ARC-Onderstepoort Veterinary Institute, unpubl. data 2002). The majority of poisonings occurred in dogs and cats, but sporadic occurrences in cattle, birds, monkeys and antelope were also reported. However, the official records reflect only a small percentage of the actual cases, as many poisonings are not reported nor samples submitted to confirm a diagnosis, as the presence of the typical round, black granules in the gastrointestinal tract or vomitus practically confirms a tentative diagnosis.

Aldicarb, an oxime carbamate insecticide, nematocide and acaricide (molecular weight = 190.3 daltons) is registered in terms of the Fertilisers, Farm Feeds, Agricultural and Stock Remedies Act (Act 36 of 1947) for the control of agricultural pests in South Africa. It is one of the most toxic carbamates, with an oral LD_{50} of less than 1 mg/kg in the rat and a dermal LD_{50} of 20 mg/kg[21]. Its mechanism of action is via inhibition of the enzyme

acetylcholinesterase, responsible for catabolism of the neurotransmitter acetylcholine, resulting in the accumulation of the latter at postsynaptic cholinergic receptor sites. Muscarinic effects include hypersalivation, lacrimation, urination, diarrhoea, bradycardia, bronchoconstriction with excess bronchial secretions and miosis. Nicotinic effects manifest as tremors, muscle stiffness, weakness and paralysis[8,9]. The muscular hypertonia, tremors and convulsions can lead to exertional rhabdomyolysis[17]. Mortalities are commonly attributed to respiratory failure[9]. In companion animals it would appear that mild intoxication can be successfully treated, but more severe cases usually die, despite intensive treatment. The most important treatment is repeated parenteral administration of atropine, which acts as a competitive antagonist of acetylcholine at the muscarinic receptors. Atropine has no effect on nicotinic receptors and will not counteract muscle tremors, weakness or paralysis[3,9].

Decontamination procedures such as induction of emesis and/or gastric lavage, in conjunction with adsorbents such as activated charcoal can be effective to limit absorption of the toxin from the gastrointestinal tract. Additional supportive treatment must be given until the animal has eliminated the poison. Most cases arrive at veterinary practices only when signs of intoxication become evident. If an additional agent, which will bind or complex aldicarb in the circulation can be administered as soon as clinical signs become evident, mortalities will most probably be curtailed.

Cyclodextrins are capable of binding toxins of low molecular weight and are

toroidal ('doughnut-shaped') oligosaccharides produced from starch by the bacterium *Bacillus macerans*[5,7] and contain 6, 7 or 8 α-D-glucopyranosyl residues referred to as α, β and γ-cyclodextrins, respectively[5]. They have a hydrophilic exterior, but a hydrophobic interior cavity and owing to this cyclic structure, cyclodextrins have the ability to forminclusion complexes with many lipophilic molecules[15].

Cyclodextrins may host a great variety of molecules, having the size of 1 or 2 benzene rings, or even larger ones, which have a side chain of comparable size to form crystalline inclusion complexes[12]. The complex renders lipid-soluble molecules relatively more watersoluble and aids renal excretion. Molecules that are hydrophobic, or have a fatty acid side chain, will partition into the cavity of the cyclodextrins, forming a molecular complex, changing the physical and biochemical properties of the 'guest' molecule[22].

This feature has been used to treat mice suffering from experimental hypervitaminosis A. Vitamin A and other retinoids are known to form complexes with cyclodextrins[19]. β-Cyclodextrin administered at 470 mg/kg over 24 hours intravenously, had been used to treat a child suffering from hypervitaminosis A, with empirical success, although no conclusion could be made from only 1 case[4]. Hydroxypropyl-_-cyclodextrin has been shown to protect sheep against tunicaminyluracil (corynetoxin) toxicity (annual ryegrass toxicity), when administered intraperitoneally at 100 mg/kg twice daily[22].

In an *in vitro* study to evaluate the inactivation of the organophosphorous nerve agents it was observed that cyclodextrins catalysed the inactivation of the compounds sarin and soman, but did not inactivate tabun and VX. Furthermore, sarin and soman showed greater affinity for β-cyclodextrins than for the α- or γ-cyclodextrins[7].

β-Cyclodextrin has been used to complex the E-isomer of mevinphos, an organophosphor insecticide2 and also enhanced biological detoxification of industrial waste-waters containing numerous pesticides[18]. α-dicyclopropylmethane was complexed with γ-cyclodextrin to decrease the acute oral toxicity of the parent compound in rats[16].

Several acute toxicity studies of cyclodextrins have been conducted in mice, rats and dogs[1,12,24]. The parenteral LD50 of γ-cyclodextrin in mice exceeds 4000 mg/kg and in rats more than 2400 mg/kg[12]. Inmice single intraperitoneal injection of 10 000 mg/kg and intravenous administration of 2000 mg/kg hydroxypropyl-β-cyclodextrin did not induce mortalities[12].

β-Cyclodextrin and hydroxypropyl-β- cyclodextrin distribute rapidly after intravenous administration to rats[11]. Both are eliminated by glomerular filtration and over 90 % of the dose is recovered unchanged in the urine within 24 h of administration[11].

The objective of the current experiments was to determine if hydroxypropyl-β-cyclodextrin (30 times more water-soluble than β-cyclodextrin)[20] is

effective in the treatment of rats poisoned with aldicarb.

4.3. MATERIALS AND METHODS

4.3.1. Chemicals

Aldicarb (99.9 % pure, Riedel-de Haën) was dissolved in sterile water (0.1 % m/v) before dosing and hydroxypropyl-β- cyclodextrin (97+ %,Acros Organics) was dissolved in distilled water (10 % m/v) before intravenous administration.

4.3.2. First experiment

Male Sprague-Dawley rats (*n* = 12), weighing 280–305 g, were randomly divided into 3 groups. Four rats in the positive control group each received an estimated lethal dose of aldicarb (1.5 mg/kg) by oral gavage. Six rats received the same aldicarb dose orally of which 3 were injected with hydroxypropyl-_-cyclodextrin (200 mg/kg) in the caudal vein immediately before aldicarb administration and the other 3 rats were dosed with aldicarb just prior to intravenous cyclodextrin administration. Two rats, serving as negative controls, only received 200 mg/kg cyclodextrin intravenously.

4.3.3. Second experiment

Male Sprague-Dawley rats (*n* = 12), weighing 170–215 g, were randomly divided into 2 equal groups. Group 1 rats were first dosed with aldicarb, at 1.5 mg/kg, orally and immediately thereafter were injected with 250 mg/kg cyclodextrin intravenously. Group 2 rats received 250 mg/kg cyclodextrin intravenously immediately prior to being dosed with aldicarb (1.5 mg/kg) per os.

4.3.4. Third experiment

Male Sprague-Dawley rats (*n* = 14), weighing 245–300 g, were randomly divided into 2 groups. Control rats (*n*=7) received a slightly lower dose of 1.3 mg/kg aldicarb orally and the rats in the experimental group (*n* = 7) received the same dose of aldicarb, by gavage, immediately after the intravenous administration of cyclodextrin at 250 mg/kg. The lower, but still estimated fatal, dose of 1.3 mg/kg aldicarb was decided upon to allow slightly more time for the hypothesized cyclodextrin-aldicarb complex formation.

4.3.5. Data analysis

The data obtained from the experimental groups were captured and analysed with the *t*-test and the Mann-Whitney rank sum test for statistical differences (Sigma Stat, Jandel Scientific, San Rafael, CA).

4.4. RESULTS

4.4.1. First experiment

In the control rats, only dosed with aldicarb, the mean survival time was 28.25 ± 7.52 minutes and in the experimental group (aldicarb plus cyclodextrin) mean survival time was 35.16 ± 16.26 minutes. No statistical significant differences occurred between groups. Although the survival times of 4 rats in the experimental group were similar to the control group, 2 of the 3 rats, which first received cyclodextrin and then aldicarb, noticeablysurvived longer (Figure 13). The 2 ratsonly receiving cyclodextrin, survivedwithout exhibiting any adverse effect.

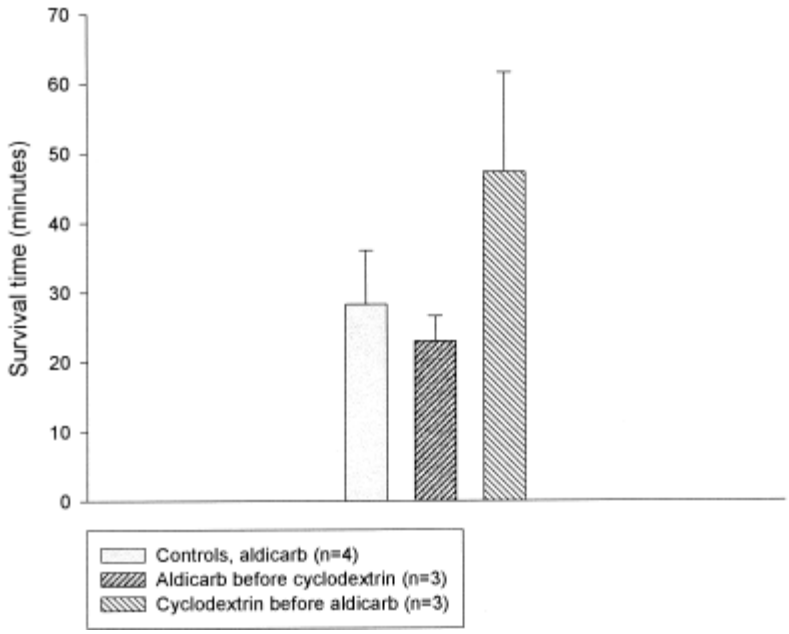

Figure 13: **Survival times of rats dosed with aldicarb (1.5 mg/kg) with and without hydroxypropyl-_-cyclodextrin (200 mg/kg) administered intravenously**.

4.4.2. Second experiment

Four rats that were first injected with cyclodextrin and then dosed with aldicarb survived longer than any of the rats receiving aldicarb before intravenous administration of cyclodextrin. The mean survival time of the group of rats that were first dosed with aldicarb followed by cyclodextrin was 34 ± 7.77 minutes and for the group that received cyclodextrin before aldicarb it was 57.5±24.52 minutes (Figure 14); these differences were statistically significant ($P < 0.05$).

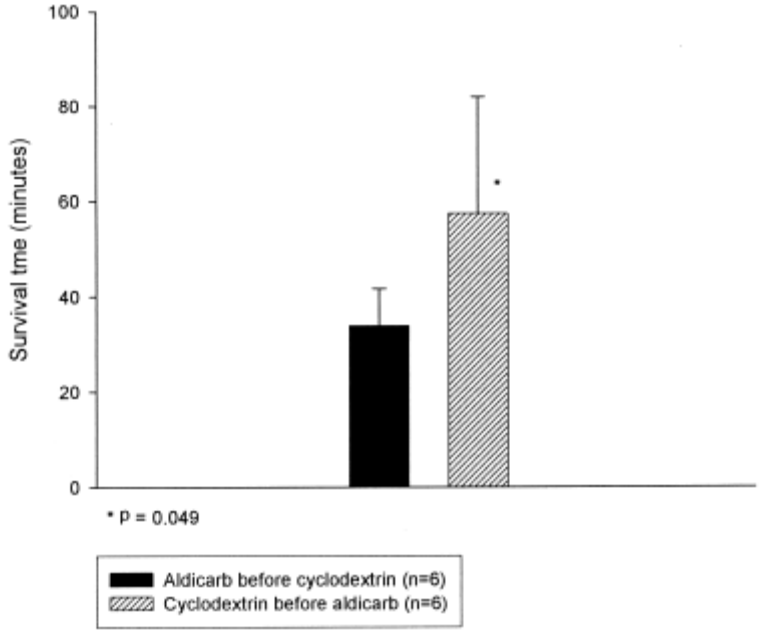

Figure 14:**Survival times of rats dosed with aldicarb (1.5 mg/kg) before and after intravenous hydroxypropyl-_cyclodextrin (250 mg/kg).**

4.4.3. Third experiment

Statistical significant differences (*P* = 0.002) were determined in mean survival time between the control group (35.28 ± 11.78 minutes; range 20–52) and the experimental group (1896.42±2267.17 minutes; range 45–4320) (Figure 15). Six of the 7 rats in the experimental group survived longer than any of the rats in the control group. Three rats that had survived for 72 hours (4320 minutes) were euthanased (overdose of pentobarbitone administered intraperitoneally). Although they did not exhibit any clinical signs reminiscent of aldicarb poisoning at the time of euthanasia they lost between 7 and 10 % body mass and it was decided to terminate the experiment.

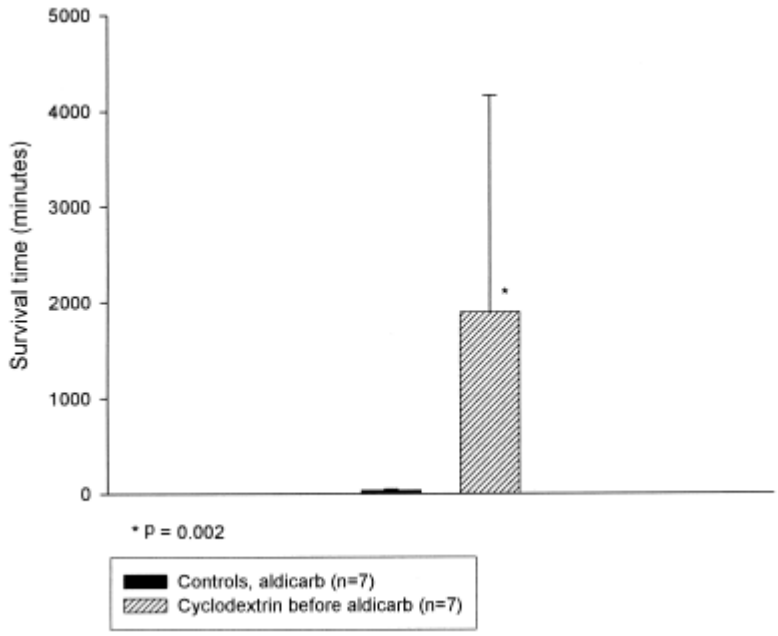

Figure 15:**Survival times of rats dosed with aldicarb (1.3 mg/kg) and the same dose of aldicarb following intravenous administration of hydroxypropyl-_-cyclodextrin (250 mg/kg).**

4.5. DISCUSSION

This study indicates that intravenous administration of hydroxypropyl-β-cyclodextrin to rats before oral dosing ofaldicarb prolongs survival and it would appear that hydroxypropyl-β-cyclodextrin complexes aldicarb to some extent. However, this will limit the therapeutic use of cyclodextrins because pets will ingest aldicarb and treatment will only commence once clinical signs become apparent. Possible explanations for the unsatisfactory results of hydroxypropyl-β-cyclodextrin in these experiments are the exceptionally high doses of aldicarb administered to the rats and the fact that aldicarb is rapidly metabolised *in vivo* to aldicarb sulphoxide and sulphone, more water-soluble compounds[23], which arguably bind poorly to cyclodextrin.

Larger doses of hydroxypropyl-_-cyclodextrin, being a relatively safe compound, could also be evaluated as the increase in dose improved survival times in rats. The intravenous doses of hydroxypropyl-β-cyclodextrin, administered in this study, were up to 250 mg/kg and deaths were attributed to aldicarb and not cyclodextrin.

Administration of atropine remains the most important treatment for carbamate and organophosphor poisoning; however, hydroxypropyl-β-cyclodextrin may be included as a supportive treatment. Considering the safety of cyclodextrins and the ability to complex organophosphors[2,7] it might contribute to the survival of organophosphor-poisoned pets.

4.6. ACKNOWLEDGEMENTS

The financial contributions received from Bayer CropScience and the Faculty of Veterinary Science is gratefully acknowledged. The authors thank Mr M Smuts and Mr P Selahle of the University of Pretoria Biomedical Research Centre for assisting with the dosing and intravenous injections of the rats and the use of the facilities. Mrs M Mülders is thanked for dissolving the aldicarb and cyclodextrin. Research reported here emanates from Project number 36-5-586, approved by the Animal Use and Care and Research Committees of the Faculty of Veterinary Science, University of Pretoria.

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Aldicarb, a highly effective pesticide, is also extremely toxic. The correct usage of aldicarb on various crops provides major agricultural-economic advantages. However, the illegal and criminal use of aldicarb has become a persistent problem throughout South Africa where current law enforcement is ineffective to decrease the risk to an acceptable level.

To prevent accidental poisoning the manufacturer of "*Temik*" has incorporated various safety measures with respect to the dry granular formulation. Each granule, containing 15 % aldicarb, is coated with an outer layer of "*Bitrex*" (denatonium benzoate), a strong, bitter agent to discourage ingestion. An anti-static flow agent is included to decrease skin contact, thus preventing percutaneous absorption.

A survey was conducted in Gauteng and although it may not accurately reflect the situation in the rest of the country, it is a good indicator of the occurrence of aldicarb poisoning in pets. Veterinarians in the greater Pretoria area reported that most cases in dogs occur in the eastern suburbs and parts of Centurion. The majority of cases observed in dogs in the greater Johannesburg area occur in the central and eastern areas and on the East Rand. The incidence of poisoning in cats follows a different pattern and they are probably often inadvertently poisoned when bait is placed out.

The ingestion of "*Temik*" granules will induce toxicosis and animal owners should not attempt to administer any treatment, but should rather present the animal to the nearest veterinary clinic as soon as possible. Based on the responses received from the veterinarians, it is clear that the success of treatment is directly proportional to the time elapsed before treatment is initiated. The rapid course of intoxication also became evident in the rat-cyclodextrin study, where some control rats died within 20 - 30 minutes, after receiving a lethal oral dose of aldicarb.

The majority of veterinarians always observed tremors and salivation. Emesis, seizures, bradycardia, dyspnoea and miosis were also often seen. Although diarrhoea was frequently observed, excessive urination was only occasionally recorded. In addition, paresis or paralysis was also noted. As can be expected, the majority of veterinarians always administer atropine to alleviate the muscarinic signs. Intravenous fluid and electrolyte therapy and the oral administration of activated charcoal are also used frequently. The enzyme reactivators and diphenhydramine are seldom administered. Diphenhydramine will alleviate the muscle trembling and is an effective treatment, which more veterinarians could utilize.

When evaluating the efficacy of cyclodextrin in aldicarb poisoning, the survival time increased when rats, dosed with aldicarb, also received cyclodextrin intravenously. This study indicates that intravenous administration of hydroxypropyl-β-cyclodextrin to rats before oral dosing of aldicarb prolongs survival and it would appear that hydroxypropyl-β-cyclodextrin complexes

aldicarb to some extent. The slightly disappointing efficacy of hydroxypropyl-β-cyclodextrin's in complexing aldicarb could be as result of exceptionally high doses of aldicarb administered to the rats and the fact that aldicarb is rapidly metabolised *in vivo* to aldicarb sulphoxide and sulphone, more water-soluble compounds, which arguably bind poorly to cyclodextrin.

To improve the likelihood of aldicarb complexation by hydroxypropyl-β-cyclodextrin, it was decided to increase the intravenous dose from 200 mg/kg to 250 mg/kg. The higher dose increased rat survival time in the second and third experiments. A trend for improvement following the increase in the dosage suggests that even larger doses of hydroxypropyl-β-cyclodextrin could also be evaluated, as it is a relative safe compound. Although atropine remains the most important treatment for carbamate poisoning, hydroxypropyl-β-cyclodextrin may be included as adjunctive therapy.

It was concluded that the full extent of the incidence of aldicarb poisoning in South Africa would never be fully known, because not all incidents are reported to the police. Owners should be encouraged to report every case to the South African Police Service. In addition, not all poisoned pets are presented for treatment nor are every carcass submitted for necropsy to veterinarians and only a minority of cases are confirmed by laboratory chemical analysis.

CHAPTER 6

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APPENDIX A

QUESTIONNAIRE

Cover letter

Dear colleague,

The Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria is currently conducting a study to find additional treatments for aldicarb poisoning in companion animals. The numbers of confirmed cases most probably do not accurately reflect the number of cases seen by private veterinarians. The problem of dogs being intentionally poisoned, seems to be on the increase, but no knowledge exist on the other aspects e.g. suburbs with highest numbers, efficacy of current treatments and percentage of successful treatments. This survey is independent from the one conducted by the Department of Agriculture and will form part of a postgraduate study. It will be appreciated if you can spend five to ten minutes to complete our questionnaire. Answers will be kept strictly confidential.

QUESTIONNAIRE: ALDICARB POISOINING OF COMPANION ANIMALS IN GAUTENG

Name of Veterinary surgeon:
_____

Name of practice: _____

Suburb/Town: _____

1. a. Total number of clinical cases presented to your practice during 2003:

Dogs	Cats

b. Estimated total number of all cases of **organophosphore** and **carbamate** poisonings (acetylcholinesterase inhibition) seen:

Dogs	Cats

c. Total number of suspected cases of **aldicarb** (Temik®, Sanacarb®) poisoning (black granules in bait, vomitus or gastric contents)

Dogs	Cats

d. Total number of confirmed **aldicarb** cases (specimens submitted to the Toxicology Division, OVI and other laboratories)

Dogs	Cats

For the remaining questions, please mark the blocks with an X to indicate the applicable answers. If you want to supply additional information, it can be done in the space underneath each question.

2. Treatment regimen for suspected aldicarb poisoning:

a) Atropine

Always	Often	Sometimes	Never
☐	☐	☐	☐

b) Induce emesis

Always	Often	Sometimes	Never
☐	☐	☐	☐

c) Activated charcoal

Always	Often	Sometimes	Never
☐	☐	☐	☐

d) Gastric lavage

Always	Often	Sometimes	Never
☐	☐	☐	☐

e) Fluid therapy

Always	Often	Sometimes	Never
☐	☐	☐	☐

f) Enzyme reactivators (e.g. Toxogonin)

Always	Often	Sometimes	Never
☐	☐	☐	☐

g) Oxygen therapy

Always	Often	Sometimes	Never
☐	☐	☐	☐

h) Diphenhydramine

Always	Often	Sometimes	Never
☐	☐	☐	☐

i) No treatment (eg. elects euthanasia)

Always	Often	Sometimes	Never
☐	☐	☐	☐

_____

_____

j) Any practical proposals / experience, which could assist with the more effective treatment of poisoned animals?

_____

_____

_____

k) Any practical proposals / experience regarding preventive actions which could assist the animal owner?

_____

_____

l) What is the approximate duration of treatment of the animal before it died/survived?

_____

3. Clinical signs observed

a) Tremors

Always	Often	Sometimes	Never
☐	☐	☐	☐

b) Salivation

Always	Often	Sometimes	Never
☐	☐	☐	☐

c) Emesis / Vomition

Always	Often	Sometimes	Never
☐	☐	☐	☐

d) Miosis

Always	Often	Sometimes	Never
☐	☐	☐	☐

e) Diarrhoea

Always	Often	Sometimes	Never
☐	☐	☐	☐

f) Urination

Always	Often	Sometimes	Never
☐	☐	☐	☐

g) Dyspnoea / Respiratory distress

Always	Often	Sometimes	Never
☐	☐	☐	☐

h) Bradycardia

Always	Often	Sometimes	Never
☐	☐	☐	☐

i) Seizures

Always	Often	Sometimes	Never
☐	☐	☐	☐

j) Other (please give information in space below)

Always	Often	Sometimes	Never
☐	☐	☐	☐

---

4. Survival rate

a) 100 % b) 75 % c) 50% d) 25% e) < 25%

---

5. Average cost to client

a)< R500 b) R500 c) R750 d) R1000 e) R1500 f)>R1500

---

6. "Seasonal" occurrence

a) Summer b) Winter c) Autumn d) Spring e) Throughout year

---

7. Were post-mortems performed?

Always ☐ Often ☐ Sometimes ☐ Never ☐

Please describe the most common macroscopic lesions observed

_____

_____

_____

8. In your opinion, is there an increase in suspected cases in your practice?

a) Yes ☐ b) No ☐

_____

_____

_____

_____

9. Reason given by owner why the pet was poisoned (Indicate a percentage e.g. 50%)

a. Nuisance/Neighbours ☐

b. Criminal intent ☐

c. Other (Specify) ☐

_____

_____

_____

10. How soon after poisoning is the animal usually presented?

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11. Which source of reference do you prefer to utilise in the case of poisonings?

Other Veterinary Surgeons/Persons/websites/Poisoning centres/ other/own personal experience?
