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CHOLECALCIFEROL: A UNIQUE TOXICANT FOR RODENT CONTROL

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ABSTRACT: Cholecalciferol is an acute (single-feeding) and/or chronic (multiple-feeding) rodenticide toxicant with unique activity for controlling commensal rodents including anticoagulant-resistant rats. Cholecalciferol differs from conventional acute rodenticides in that no bait shyness is associated with consumption and time to death is delayed, with first dead rodents appearing 3-4 days after treatment.

INTRODUCTION

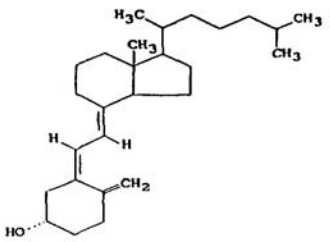
It has been well documented over the past two decades that commensal rodents are becoming more difficult to control with anticoagulant rodenticides (Jackson and Kaukeinen 1972). House mice have historically been more difficult to control with anticoagulants, including even the newer "second-generation" products, as they all function through the same biochemical pathway (Bull 1983).

Even before the introduction of newer, more potent, anticoagulants, research had been started by Bell Laboratories, Inc., to search for a replacement rodenticide that possessed many of the beneficial attributes of anticoagulants but with a mode of action unique to the compound.

This work led to the careful review of cholecalciferol (Vitamin D_3) as a possible rodenticide candidate.

PHYSICAL CHARACTERISTICS

Technical cholecalciferol is an amber crystalline solid. Cholecalciferol is practically insoluble in water, soluble in the usual organic solvents, and slightly soluble in vegetable oils.



$C_{27}\ H_{44}\ O$ Molecular weight 384.62

9,10-Secocholesta - 5,7,10(19)-trien-
3B - ol; activated 7 - dihydrocholesterol

EFFICACY

In order to determine the efficacy of cholecalciferol on target commensal rodent species, toxicity, palatability and efficacy to target species needed to be established.

Acute Oral LD_{50}

Cholecalciferol was dissolved in corn oil and administered to the Norway rat and house mouse (Table 1). Oral toxicity values indicate cholecalciferol is toxic to target species with house mice being slightly more susceptible than Norway rats.

Table 1. Acute Oral LD_{50} (mg/Kg) for Cholecalciferol.

Species	Strain	Sex	LD_{50}
Mus musculus	ICR	M/F	42.5 mg/kg
Rattus norvegicus	Sprague-Dawley	M/F	43.6 mg/kg

Efficacy/Palatability Studies

Choice-feeding studies were conducted following the EPA acute dry bait test protocol against Norway rats, roof rats and house mice. Concentrations of 750 ppm (.075%) were utilized based upon prior no-choice feeding trials (Kassa, Jackson 1978). The choice-test results are summarized in Table 2.

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Mortality of 100% occurred with only three days' exposure to cholecalciferol. Average days until death ranged from 3.9 to 6.1 in mice, 3.3 to 4.7 in Norway rats, and 10.2 in roof rats. Cholecalciferol was readily accepted by the test animals, even when a palatable placebo test diet was available. There is no taste aversion to cholecalciferol and bait shyness is not apparent due to delayed toxic effects.

Table 2. Choice efficacy studies with cholecalciferol bait.

Species	Strain	Conc. (ppm)	No. dead/ No. tested	Avg. days until death	% acceptance of cholecalciferol bait
R. norvegicus	Wistar	750	5/5	3.5	39.8
R. norvegicus	Wistar	750	10/10	3.7	61.1
R. norvegicus	Wistar	750	10/10	3.4	53.7
R. norvegicus	Wistar	750	10/10	4.7	43.1
R. norvegicus	Wistar	750	10/10	3.9	47.3
R. norvegicus	Wistar	750	10/10	3.3	50.4
R. norvegicus	Wistar	750	10/10	3.5	43.3
R. norvegicus	Wistar	750	10/10	3.4	49.8
R. norvegicus	Holtzman	750	10/10	4.4	41.5
R. norvegicus	Wistar	750	10/10	3.6	52.8
R. norvegicus	Wistar	750	10/10	3.7	45.8
R. norvegicus	Wistar	750	10/10	3.5	38.1
R. norvegicus	Wistar	750	10/10	3.3	52.8
Mus musculus	ICR	750	20/20	6.1	44.6
Mus musculus	Swiss Webster	750	10/10	5.0	52.5
Mus musculus	ICR	750	10/10	4.3	48.0
Mus musculus	ICR	750	20/20	3.9	35.3
Mus musculus	ICR	750	10/10	4.3	39.5
Mus musculus	ICR	750	10/10	4.7	31.1
Mus musculus	ICR	750	10/10	4.3	31.8
Mus musculus	ICR	750	10/10	5.5	37.1
R. rattus	Wild	750	5/5	10.2	45.7

Anticoagulant-Resistant Rats

Anticoagulant-resistant Norway rats were obtained from Dr. John Suttie, University of Wisconsin-Madison. The resistant rats were acclimated to laboratory conditions for 14 days and maintained on a diet of Purina Rat Chow.

The obtained anticoagulant-resistant rats were placed on the standard World Health Organization tests (WHO 1970) for warfarin resistance. After a 30-day cleansing period on a toxicant-free diet, the resistant rats were placed on the standard EPA acute dry bait test. Results are summarized in Table 3. Resistant rats showed no hesitation in accepting the cholecalciferol bait, killing 100% of the test animals with average day-to-death and consumption figures being similar to other choice feeding studies.

Table 3. Choice-efficacy studies with choiecalciferol bait against Warfarin-resistant (WHO standard) rats.

Species	Conc. (ppm)	No. dead/ No. tested	Avg. days until death	% acceptance of cholecalciferol bait
R. norvegicus	750	20/20	4.3	43.6
R. norvegicus	750	9/9	4.6	53.6

TOXICOLOGY

Hazard Evaluation

Working with a human vitamin gave way to a considerable amount of chronic exposure data, but very little, if any, information was available on one single acute exposure to both humans and experimental animals.

Crystalline cholecalciferol has a potency of 40,000,000 I.U./gram or 40×10^6 I.U./gram. Due to the instability of crystalline cholecalciferol, resinous material was chosen as the starting material with a potency of 28,000,000 I.U./gram or 28×10^6 I.U./gram.

Cholecalciferol in the technical form (40 x 10^6 I.U./gram) is quite toxic; however, the toxicity is reduced proportionately as the concentration of the active material is reduced.

Due to the nature of the compound and physical characteristics of not only the technical material and resinous concentrate, but also the formulation of the finished bait at 750 ppm cholecalciferol (.075%), several of the acute-hazard evaluation studies were waived by the U.S. Environmental Protection Agency. The finished bait is of low oral and dermal toxicity at concentrations tested (Table 4).

Table 4. Acute Hazard Evaluation of Cholecalciferol.

Test	100% Technical	.075%
Oral (rats) LD_{50}	43.6 mg/kg	-
Dermal (rabbits) LD_{50}	-	2,000 mg/kg.

Acute Nontarget Species Toxicity Studies

Acute-toxicity data were generated for cholecalciferol on nontarget species. It is known that Vitamin D_2, is only 1-2 percent as potent for the chick as Vitamin D_3 (Edes, personal correspondence). Because of this difference, the majority of current nontarget hazard evaluations have been conducted on avian species (Table 5). Results indicate that cholecalciferol is of low hazards to avian and canine species.

Table 5. Cholecalciferol toxicity data from acute and short-term feeding studies to nontarget species.

Species	LD_{50}/LC_{50}
Dog - oral	88 mg/kg.
Mallard duck - oral*	2,000 mg/kg.
Mallard ducklings - dietary*	4,000 ppm
Bob white quail - dietary*	2,000 ppm

* studies conducted on 30% Concentrate (12 x 10^6 I.U./Gram).

Secondary Toxicity

Groups of Norway rats (Wistar strain) were fed free choice diets of .075% cholecalciferol bait versus EPA Challenge Diet following the TSD Designation 1.209(9-1-76) (Acute Dry Bait test protocol). It was determined that by following this test protocol would best simulate actual field conditions. All rats died from test diet ingestion, were skinned and the carcasses ground. The ground rats were packaged, labeled and frozen prior to being offered as dog food.

Six beagle-type dogs (sexes equal) were acclimated to laboratory conditions for nine days prior to the initiation of the project. Test animals were fasted for 24 hours prior to test. All dogs were maintained on the test diet for 14 days or until death occurred. All test animals survived the 14 day no-choice feeding regime showing no signs of cholecalciferol intoxification or hypervitaminosis D. No pathologic abnormalities were noted. It was concluded that dogs consuming rats poisoned by cholecalciferol did not receive a sufficient amount of toxicant to cause hypervitaminosis D even when fed a diet of poisoned rats exclusively for 14 days. It is reasonable to assume, therefore, that cholecalciferol does not pose a potential secondary hazard to canine species.

MODE OF ACTION

Cholecalciferol enters the body via oral ingestion and first accumulates in the liver. Here, the first metabolic change begins. An enzyme in the liver adds a hydroxl group(-0H) to the 25th carbon atom which is located in the side chain of the molecule (DeLuca 1979). The product, commonly referred to as 25-OH-D_3, is released into the blood stream where it is present in concentrations approximately 10 times the revels of Vitamin D. When there is a metabolic demand for calcium or phosphorus (blood levels fall), 25-OH-D_3, is metabolized in the kidney where another enzyme adds a second hydroxyl group at the number 1 carbon atom. This is 1,25-$(OH)_2$ D_3, and is considered to be the hormonal form of Vitamin D. It would be incorrect to imply that all 25-0H-D_3 is converted to 1,25-$(OH)_2$ D_3. Under physiological conditions where relatively little calcium is needed, such as with mature animals where maintenance is the factor, another kidney enzyme is activated to convert 25-0H-D_3 to 24,25-$(OH)_2$ D_3. This seems to be part of a slowdown or shutoff mechanism for calcium mobilization.

Major functions of Vitamin D in animal nutrition are known. Many critical reactions in the body require calcium. In addition to forming new bone, calcium is needed for the formation of egg shells, milk production, blood clotting, neuromuscular action and a host of other functions. It is not surprising, therefore, that the pool of calcium circulating in the blood is very carefully regulated. In most nonavians, changes of more than 10-15% can be disastrous to the living system. Consequently, a sophisticated system involving the intestines, kidneys and skeleton is primarily regulated by the hormones: parathyroid hormone (PTH), calcitonin, and 1 ,25-$(OH)_2$ D_3 that generally keep blood calcium levels within 2-3% of normal. If calcium levels should fall Below normal, the kidney is stimulated by PTH to produce 1,25-$(0H)_2$ D_3.

As 1,25-$(OH)_2$ D_3 Do levels rise, cells in the small intestine are stimulated to absorb more calcium and phosphorus. Bone is also mobilized in the presence of PTH and l,25-$(OH)_2$ D_3 to release calcium and phosphorus into the blood stream. These factors along with increased kidney reabsorption of calcium tend to raise the; blood calcium and phosphorus levels. If calcium levels in blood rise too much too rapidly, and the normal regulation by hormone activity fails to counteract the process, the net result is a system failure responsible for resulting calcification diseases including blockage of the circulatory system. This is the fatal action demonstrated in rodents that have consumed large doses of cholecalciferol as a rodenticide. Mortality in all animals is exhibited, but rodents succumb to lower doses than other animals simply because of their small size. The effect of raised blood calcium levels in mammalian and avian species is effectively regulated by the thyroid hormone, calcitonin, that is released to counteract the process initiated by PTH and 1,25-$(OH)_2$ D_3. Hormones react rapidly in mammalian and avian species to keep blood and other critical fluids fully saturated with calcium at normal levels to enable bone formation and other critical calcium reactions to occur on demand.

CONCLUSION

Cholecalciferol has been proven to be toxic and effective to target species, yet relatively safe to nontarget species if used according to label directions.

Due to cholecalciferol's unique mode of action, the product looks promising for use as a commensal rodent control product, but due to its unique attributes (i.e. no taste aversion, delayed toxic effect) and vitamin status, future rodenticide applications for field rodents look promising.

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