CHRONIC TOXICITY TESTING OF AN ODOR-CONTROL PRODUCT APPLIED TO PET ANIMAL BEDDING

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1.0 INTRODUCTION

More than 50% of American households have pets, including a growing portion of rodents, rabbits and birds. Physiological functions in animals are a complex manifestation of the dynamic interaction of genetic and environmental backgrounds. These functions are integrated into a delicately balanced biological system that is directly influenced by the physical, chemical and microbial aspects of the animals' environment. Bedding material is an important, controllable environmental factor that can have a profound effect on pet animal health. Regardless of whether bedding is used on solid floors, in pens or cages, it should provide a surface that is hygienic and which fulfills both physical and chemical requirements essential to the health and well-being of the animals. Bedding should be dust-free and suitable to walk upon and to nest in, i.e., it has to be soft. It must be capable of absorbing odors and liquids, two processes facilitated by a large surface area. Ready availability of a renewable, biodegradable resource is another highly desirable characteristic in terms of being environmentally friendly. Studies have shown that animal bedding material differs in its ability to control odors, produce dust and absorb liquids.

1.1 Objective

The purpose of this study was to establish a baseline safety assessment profile for a proprietary odor-control product manufactured for caged pet rodents (hamsters, mice), rabbits, ferrets and psittacine (cockatiels) and passerine (Zebra finches birds. To examine the safety of this formulation applied to dried comminuted corn cob bedding for pet use, 60-90 days of continuous exposure to regularly changed bedding material was performed for each of four mammalian and two avian species. Specifically, mice, hamsters, ferrets, rabbits, finches and cockatiels were housed in cages containing bedding material supplied by Green Pet Products, Incorporated, the study sponsor.

2.0 MATERIALS AND METHODS

An odor-control product applied to crushed corn cobs was tested for chronic toxicity (90 days) using four mammalian and two avian species; specifically, mice, hamsters, ferrets, rabbits, cockatiels and finches. Following a seven-day acclimation period, animals were weighed and randomly assigned to experimental and control groups. Cages were supplied by the Office of Laboratory Animal Medicine, University of Missouri, except the bird cages which were supplied by Green Pet Products. Caging met or exceeded the NIH guide for the care and use of laboratory animals.

2.1 General Protocol

Female mammalian species were selected as test subjects, except in the case of ferrets where males were used, and placed in contact with untreated (10 controls) or odor-control treated bedding (10 experimentals) for 60-90 days. Male ferrets were used to avoid the problem of endogenous estrogen intoxication associated with unbred female ferrets. Animals were observed daily, and records of clinical observations were maintained with particular attention given to ventral body surfaces and feet (areas in the most intimate contact with bedding material). Clinical observations of attitude, appetite, conditions of integument and eyes, and overt signs of illness were conducted daily, Monday through Friday. Each animal was weighed weekly (mammals) or biweekly (avian) during the exposure period to monitor growth and body weight (a sensitive index of toxicity).

Each cage had bedding material applied to a depth of about 2.5 cm. Bedding was changed at least once per week, and subjective evaluation (olfactory sensing of ammonia) of odor was recorded. All animal species were fed appropriate commercial lab animal diets and had access to tap water, *ad libitum*. All species were housed in American Association of Laboratory Animal Scientists (AALAS) certified, temperature- and humidity-controlled animal rooms. Daily high and low room temperatures were recorded for each room. All mammalian species were on a 12:12 hour light:dark cycle without twilight. Birds were kept on an 8:16 hour, light:dark cycle to control aggression and limit breeding behavior. After a maximum of 90 days (60 days for 10 additional finches and 60 days for five additional mice), each group of animals was humanely euthanized by carbon dioxide inhalation, and a gross necropsy was performed by a board-certified veterinary pathologist. Brain, heart, liver, lung, kidney, intestine and skin from the ventral aspect of the abdomen were fixed in 10% buffered formalin, sectioned, H&E stained and evaluated microscopically. Histological findings were scored and summarized for each animal.

Control animals were placed on untreated corn cob bedding, and experimental groups were placed on corn cob bedding treated with Green Pet Products proprietary odor suppressant. All animals were in direct contact with the bedding at all times to maximize exposure, except the birds, which were allowed perches to rest upon.

Control animals included 10 per species, except for the nine finch controls. The following table delineates the precise numbers of each animal.

Species	**Number of Animals**	**Treatment**
Ferrets	10	Control*
	10	Experimental**
Hamsters	10	Control
	10	Experimental
Mice	10	Control
	15	Experimental
Rabbits	10	Control
	10	Experimental
Cockatiels	10	Control
	10	Experimental
Finches	9	Control
	21	Experimental

*Untreated Green Pet Products Corn Cob Bedding
**Odor-Control Treated Green Pet Products Corn Cob Bedding

Animals were caged as follows: five finches/cage, two cockatiels/cage, five mice/cage, two hamsters/cage, one rabbit/cage and two ferrets/cage. Caging was consistent with NIH guidelines for the care and use of laboratory animals.

All animals were observed daily for clinical abnormalities and weighed weekly. With the exception of the rabbits, bedding was changed at least one per week, and a subjective evaluation of the odor (olfactory detection) was recorded. Bedding in the rabbit cages was changed on an every-other-day basis and wet spots removed daily. After 60 days of continuous exposure to the bedding, 10 finches and five mice were euthanized on day 90 and necropsied. Brain, heart, liver, lung, kidney, intestine and skin were fixed in formalin, sectioned, H&E stained, evaluated histologically.

3.0 RESULTS

With respect to body weight gain and maintenance, clinical evaluation, gross necropsy findings and histological evaluation, there were no detectable differences between control (untreated) and experimental (treated bedding) groups. There were also no detectable abnormalities or findings inconsistent with expected normal background for any of these species. Ammonia levels in cages containing treated or untreated bedding were subjectively evaluated using olfactory detection. No ammonia was detected in cages containing treated bedding. Detailed data for individual animals can be found in the accompanying hardcopy and/or the designated Excel® file (xls extension files,

version 5.0). Health evaluation forms were constructed in Word Perfect 6.0, while memorandums were constructed in Microsoft Word 6.0. Hardcopy files are categorized by animal species and include daily observation and/or daily health evaluation forms, and histological evaluation forms labeled pathology. Files labeled necropsy forms include gross examination results of individual animals. Hardcopies of weight changes are included in the animal weights file category.

4.0 CONCLUSIONS

Based on the monitored experimental parameters (clinical observations, body weight changes, and gross and microscopic evaluation of selected tissues), Green Pet Products odor-control bedding is safe for a variety of pet animal species. Subjective evaluation of the bedding material also revealed suppression of ammonia production and minimal to nondetectable dust production from agitated material.