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Calcium lignosulphonate: Re-evaluation of relevant endpoints to re-confirm validity and NOAEL of a 90-day feeding study in rats

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ABSTRACT

A 90-day feeding study in Han/Wistar rats with calcium lignosulphonate was evaluated by the EFSA. The study was considered to be inadequate due to potentially impaired health status of the animals based upon a high incidence of minimal lymphoid hyperplasia in mesenteric/mandibular lymph nodes and Peyer's patches, and minimal lymphoid cell infiltration in the liver in all animals. The EFSA Panel further disagreed with the conclusion that the treatment-related observation of foamy histiocytosis in mesenteric lymph nodes was non-adverse and asked whether this observation would progress to something more adverse over time. A PWG was convened to assess the sections of lymph nodes, Peyer's patches and liver. In addition, all lymphoid tissues were re-examined. The clinical pathology and animal colony health screening data were re-evaluated. The question whether the foamy histiocytosis could progress to an adverse finding with increasing exposure duration was addressed by read-across. In conclusion, the animals on the 90-day feeding study were in good health, the study was adequate for safety evaluation, and the foamy histiocytes in the mesenteric lymph nodes were not considered adverse, but rather an adaptive response that was considered unlikely to progress to an adverse condition with time. The NOAEL was re-affirmed to be 2000 mg/kg bw/d.

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1. Introduction

Calcium lignosulphonate is an amorphous yellow–brown to brown polymer derived from lignin, without a well-defined structural or molecular formula and with an average molecular weight between 40,000 and 65,000 Da. The product is used as a carrier for carotenoids in feed preparations.

In vitro and in vivo assays have shown calcium lignosulphonate to be poorly absorbed by the oral route. An Ames test and an in vitro chromosome aberration test were both negative. A developmental toxicity study in rats showed no adverse effects at the limit dose of 1000 mg/kg bw/d. Further, minimal focal to multifocal chronic inflammation was seen in the rectum of male rats at a dose of 4000 mg/kg bw/d for 28 days but not at the next lower dose of 1500 mg/kg bw/d. In the subsequent 90-day study (see below) no effects were seen in rectum. This set of studies had been evaluated by the European Food Safety Authority (EFSA, 2010).

A report of a 90-day subchronic toxicity study with a 28-day recovery period in Wistar rats of calcium lignosulphonate was included in the submission for evaluation by EFSA. In this study treatment-related foamy histiocytosis was seen in the mesenteric lymph nodes. EFSA determined that the study was inadequate for safety evaluation purposes and rejected the study based on the

Abbreviations: BALT, bronchial associated lymphoid tissue; EFSA, European Food Safety Authority; H&E, hematoxylin & eosin; NALT, nasal associated lymphoid tissue; NOAEL, No Observed Adverse Effect Level; NTP, National Toxicology Program; PWG, Pathology Working Group; SRBC, sheep red blood cell; STP, Society of Toxicologic Pathology; TDAR, T-cell-dependent antibody response.

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presence of minimal lymphoid (germinal center) hyperplasia in mesenteric and mandibular lymph nodes as well as in Peyer's patches (jejunum and ileum), in addition to minimal focal lymphoid cell infiltrates in the liver of all animals. This was deemed to indicate that the changes reported may be consistent with "poor health status" and "could have influenced the integrity of the study" (EFSA, 2010). EFSA further suggested that the lymphoid changes as reported by the study pathologist may also be consistent with the immune system being in "an activated state" and perhaps such constant exposure to an immunogenic compound could affect the "overall health status" (EFSA, 2011).

The EFSA Expert Panel was further concerned that the foamy histocytosis observed in the mesenteric lymph nodes may progress to something more adverse with time. Therefore, a chronic toxicity study was requested for clarification.

In this publication we present the findings from an independent re-evaluation of the issue of potentially compromised health status and adequacy of the 90-day feeding study performed with calcium lignosulphonate. The following supplemental investigations were performed: additional evaluation of the clinical pathology data, re-evaluation of all lymphoid tissues by two independent experts, re-evaluation of the tissues used by EFSA for their determination of a compromised health status by five independent experts in histopathology (Pathology Working Group (PWG)), and evaluation of the animal health certificates from the regular screening program of the performing laboratory. Whether the foamy histocytosis observed in mesenteric lymph nodes was likely to progress to an adverse lesion was also evaluated by the PWG and by a literature review considering similar poorly digestible high molecular weight substances.

2. Materials and methods

2.1. Animal study

The 90-day study with calcium lignosulphonate was conducted at RCC (now Harlan Laboratories), Study Number A29553, Itingen, Switzerland. The in-life phase was from 02 January 2006 to 02 May 2006 (termination of the recovery animals).

2.2. Guidelines and GLP status

The study procedures described in herein met or exceeded the following guidelines:

"Repeated Dose 90-Day Oral Toxicity Study in Rodents", OECD Guidelines for the Testing of Chemicals, Section 4, Health Effects, Number 408, 21 September 1998. Optional neurologic, immunologic, and reproductive endpoints were included for completeness of the safety evaluation. Fecal pH was added to determine if the size and charge of the test article had any effect on intestinal contents.

"Subchronic Toxicity Studies with Rodents", Section IV.C.4.a. and "Immunotoxicity Studies", Section V.C., Toxicological Principles for the Safety Assessment of Food Ingredients, Office of Food Additive Safety, Redbook 2000, US Food and Drug Administration, USA.

This 90-day study was conducted according to Good Laboratory Practice (Swiss Ordinance relating to Good Laboratory Practice adopted May 18th, 2005 which is based on the OECD Principles of Good Laboratory Practice 1997.

Histopathological re-evaluations of microscopic slides were done using standard methods and nomenclature (Crissman et al., 2004; Haley et al., 2005; Frith et al., 2000; Elmore, 2006a, 2006b) for findings in general toxicity studies.

2.3. Test article

Calcium lignosulphonate (CAS 8061-52-7) had a purity of 95.5% and was supplied by DSM Nutritional Products AG. Pelleted standard Provimi Kliba 3433 rat maintenance diet was used as the control diet and for preparation of the test article admixes.

2.4. Test animal treatment

A total of 124 male and 124 female HanRCC:WIST (SPF) rats obtained from RCC were allocated randomly based on their body weights to the experimental groups. The control group (Group 1) and the high dose group (Group 4) consisted each of 36 animals per sex. The low dose group (Group 2) and the mid dose group (Group 3) each consisted of 26 animals per sex. The control group received the untreated control feed. Groups 2, 3, and 4 were fed diets containing the test article at concentrations to achieve doses of 500, 1000, and 2000 mg/kg bw/d, respectively, for at least 90 days. The groups comprised 20 animals per sex, which were sacrificed after 13 weeks of treatment (Allocation A animals, Table 1). An additional 10 rats per sex were used for Groups 1 and 4 (Allocation B animals, Table 1). These animals were treated for 13 weeks and then allowed a 28-day treatment-free recovery period, after which they were sacrificed. A further six animals per sex per group were used to assess possible changes in the primary immunological response after 13 weeks of treatment (Allocation C animals, Table 1). Animals were 5 weeks old at receipt and were allowed a 7-day period for acclimatization. Animals were housed individually in Makrolon type-3 cages at standard laboratory conditions (10–15 air changes per hour, temperature of 22 ± 3 °C, and relative humidity range from 30% to 70%, 12-h fluorescent light/12-h dark cycle). Animals received community tap water and pelleted control or test diets ad libitum.

Dietary admixtures were prepared with the test article as supplied. Fresh batches of the feed pellets for this study were prepared weekly, based upon the food consumption and body weights. Control feed for the animals of Group 1 was prepared similarly, but without test item.

The animal diet was analyzed using UV/VIS spectrophotometry after derivatization and quantified by absorbance at 440 nm wave length for test article concentration accuracy, homogeneity, and stability. Stability of the lowest and highest concentrations of the test article in feed was confirmed for up to 21 days at room temperature and at −20 °C (deviation < 16.5% from mean). The test article was distributed homogenously in the diets with variations in concentrations <15.2%. The diets were accurately prepared with the test article content being in the range of ±20% of nominal content. Samples of the control group were found not to contain the test article.

2.5. Parameters evaluated

2.5.1. Survival, clinical observations, body weights, food consumption, and CNS evaluations

Animals were observed twice daily for mortality/viability. Body weights and food consumption were recorded weekly. During week 13 a modified Irwin screen test was performed in all animals of Allocation A and B. During week 17 (recovery), this was done in all animals of Allocation B. Forelimb and hind limb grip strength measurements were performed. Locomotor (decreased or increased) activity was measured during treatment week 13.

2.5.2. Clinical pathology

Blood and urine sampling was conducted after 2, 6, 13 and 17 (recovery) weeks on all Allocation A and B animals, as applicable. Blood samples for hematology as well as clinical biochemistry

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