Calcium Lignosulfonate

CAS No. 8061-52-7

Follow Up 90-day Feeding Study in Rats Addressing Findings in Mesenteric Lymph Nodes

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Document status: Final
Version: 1
Release Date: 21-April-2008

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1. Introduction and Objective

Calcium lignosulfonate is a product of the sulfite pulping process of wood. In this digestion process with aqueous calcium bisulfite solution, lignin, the biopolymer second most in abundance after cellulose, solubilizes as calcium lignosulfonate. After the digestion, the cellulose and the calcium lignosulfonate are separated by filtration. The filtrate is purified by ultrafiltration and evaporated.

No definite structure is available of the highly complex, amorphous lignin polymer, although numerous models exist. In normal (softwood) lignin, the structural monomeric elements are derived principally from coniferyl alcohol (95%), with the remainder consisting mainly of p-coumaryl alcohol-type units. These alcohols are crosslinked via a variety of different chemical bonds. The acidic sulfite pulping process modifies the lignin structure by sulfonation preferably at the α-position of the side chains of the phenylpropane units.

DSM Nutritional Products Ltd now wants to use calcium lignosulfonate as formulation aid for vitamins and nutrients in food. Therefore, several toxicological studies were performed to investigate the toxicity of calcium lignosulfonate[1] in laboratory animal amongst others a 90-day feeding study in rats.

The intention of the present document is to discuss the finding of foamy histiocytes in the mesenteric lymph nodes as observed in the 90-day feeding study in rats performed with CALCIUM LIGNOSULFONATE (Thiel et al., 2007). Foamy histiocytosis in the mesenteric lymph node was not considered adverse effect because it was not accompanied by collateral damage in the respective tissue and furthermore slight recovery after 28 days cessation was noted. The question as to whether or not this finding may progress with time will be addressed using toxicity data of comparable molecule polypentosan sulfate sodium salt (PPS). Subchronic and chronic toxicity data are available for PPS. In addition, reference is made to a polyvinylpyrrolidon copolymer (copovidone, Kollidon VA 64) for which long-term toxicity data are available.

[1] Thereafter, DSM quality calcium lignosulfonate will be designated as CALCIUM LIGNOSULFONATE. The average molecular weight of CALCIUM LIGNOSULFONATE is between 40 000 and 65 000 Da with more than 90% being within 1 000 and 250 000 Da.

2. Foamy Histiocytosis in Mesenteric Lymph Node

2.1. Calcium Lignosulfonate

A 90-day feeding study was conducted in compliance with GLP and following internationally accepted guidelines: OECD 408 and FDA Redbook guidelines (Thiel et al., 2007). CALCIUM LIGNOSULFONATE (batch no. FG-R 004, purity: 95.5%) was fed to HanRCC:WIST(SPF) rats for 90 days at dose levels of 0, 500, 1000, and 2000 mg/kg bw/day (group 1, 2, 3, and 4, respectively). Each group was divided in subgroups: Allocation A of group 1 to 4 consisted of 20 animals/sex/group and was treated for 90 days (main animals). Group 1 and 4 had additional recovery animals (10 animals/sex/group, allocation B) which were kept for additional 28 days without treatment. Allocation C of group 1 to 4 consisted of 6 satellite animals/sex/group which were used for investigation in changes in primary immune response using sheep red blood cell assay (functional testing of immune response). In addition, the distribution of leukocyte subpopulation was studied after 13 and 17 weeks in Allocation B animals using flow cytometry. Diet was prepared once weekly and offered to the animals *ad libitum*.

CALCIUM LIGNOSULFONATE was well tolerated. At the end of treatment and recovery periods, no test-item related gross lesions were recorded. Histopathological examination of the rectum showed neither indication for inflammation nor for irritation. Tubular vacuolation of the kidney was noted after treatment and recovery period in females of the mid and in both sexes of the high dose group without dose-response relationship in severity. This finding was not considered as adverse effect due to the absence of tubular damage. Large focal/multifocal aggregates of foamy histiocytes in the mesenteric lymph nodes in all treatment groups following a dose-response relationship. This finding showed minimal regression after the recovery period (see Table 2-1). No concomitant damages were observed. Therefore, this finding was not considered an adverse effect.

Table 2-1: 90-day study with CALCIUM LIGNOSULFONATE: Histopathological Findings

	Group 1		Group 2		Group 3		Group 4		Recovery Group 1		Recovery Group 4	
Finding	male	female	male	female	male	female	male	female	male	female	Male	female
Foamy histiocytosis	0/20	0/20	4/20	3/20	17/20	8/20	20/20	19/20	0/10	0/10	10/10	10/10
MeanSeverity	--	--	1.0	1.0	1.3	1.3	2.3	2.1	--	--	2.1	1.8

It is concluded that dietary administration for 13 weeks to Wistar rats produced no adverse effects up to and including the highest administered dose level. The NOAEL of the study is therefore 2000 mg CALCIUM LIGNOSULFONATE/kg bw/day.

2.2. Polypentosan Sulfate

Polypentosan Sulfate sodium salt (PPS, Elmiron®), a white powder, is a semisynthetic sulfated polyanion composed of β-D-xylopyranose residues with biological properties similar to heparin. PPS is used in the United States for the relief of urinary bladder pain associated with interstitial cystitis. The molecular weight is between 1500 and 4000 Da. Usually, 300 mg PPS / day are given to patients.

The toxicity and possible carcinogenicity of PPS was investigated within the National Toxicology Program (NTP) during the 1990's (NTP, 2004) thereby exposing Fischer 344 rats and in B6C3F1 mice via gavage with PPS for 90 days or 2 years.

Groups of 10 male and 10 female rats were administered PPS in deionized water by gavage at doses of 0, 63, 125, 250, 500, or 1,000 mg/kg, 5 days per week, for 14 weeks. No deaths were attributed to administration of PPS. Mean body weights of 125 mg/kg males were less than those of vehicle controls and the mean body weights of all dosed groups of females were greater. Hematology results indicated that PPS, at the doses selected induced a minimal erythron decrease and leukocyte and platelet count increases that may have been secondarily related to the inflammatory lesions observed in various tissues of rats. Liver and spleen weights of males administered 250 mg/kg or greater were significantly increased. Liver weights of all dosed groups of females and kidney, lung, and spleen weights of 1,000 mg/kg females were significantly increased. Histiocytic cellular infiltration, chronic active inflammation, and ulcers of the rectum occurred in most 500 and 1,000 mg/kg rats. The incidences and severities of histiocytic celluar infiltration generally increased with increasing dose, and this minimal to mild lesion was characterised by aggregates of foamy macrophages within the lamina propria that filled and distended the lamina propria and resulted in disorganisation and distortion of the mucosal crypts. The macrophages were large with abundant foamy cytoplasm due to the presence of numerous intracytoplasmic, variably sized, clear vacuoles. It was argued that the material was the test substance itself. Administration of PPS was associated with the presence of vacuolated histiocytes in the mandibular and mesenteric lymph nodes. The macrophages were again filled with foamy cytoplasm with variably sized clear vacuoles. Alveolar histiocytic infiltration of the lungs and chronic interstitial inflammation in the lungs was noted in male and female rats. Kidneys and liver at 500 mg/kg bw/day or greater displayed cytoplasmatic vacuolisation in the tubuli and midzonal area, respectively.

Groups of 50 males and 50 females were administered PPS in deionized water by gavage at doses of 0, 14, 42, or 126 mg/kg to males and 0, 28, 84, or 252 mg/kg to females, 5 days per week, for 104 or 105 weeks. Survival of all dosed groups of rats was similar to that of the vehicle control groups. Mean body weights of all dosed groups were similar to those of the vehicle controls throughout the 2-year study. Microscopically, myxomatous changes were present in the rectum of 56% of 126 mg/kg males and 83% of 252 mg/kg females. The incidences of chronic active focal alveolar inflammation of the lung were increased in all dosed groups. The incidences of histiocytic cellular infiltration of the mesenteric lymph nodes were increased in 42 and 126 mg/kg males and in 84 and 252 mg/kg females, and lymphohistiocytic hyperplasia was present in the spleen of 126 mg/kg males and 252 mg/kg females. Histopathological changes in liver and kidney were not evident. Findings in the other organs like mesenteric lymph nodes and lungs were comparable after 90-day and 2-year exposure. PPS was not carcinogenic in rats.

2.3. Copovidone

Copovidone / Kollidon VA 64 is a copolymer of vinylpyrrolidone and vinyl acetate which is used in pharmaceutical industry. A molecular weight range between 45 000 and 70 000 Da has been reported. The long-term toxicity of the material was investigated in dogs with 1-year exposure via feed (Mellert et al., 2004) at dose levels of 0, 500, 1500, and 2500 mg/kg bw/day (4 animals per sex and group). Control and highest dose group contained two additional recovery animals per sex.

Administration of Copovidone to Beagle dogs did not result in any overt clinical signs. Treatment-related effects on body weights were not noted. The only finding that distinguished the treated animals from the control animals was the presence of vacuolated histiocytes in mesenteric lymph node in 2 dogs of the high dose groups and one dog in each of the lower dose groups with dose-dependent increase in severity: moderate in the high dose group, slight in the mid and minimal in the low dose group. The vacuolated histiocytes were found in the sinusoids and trabeculae of some mesenteric lymph nodes. Staining techniques indicated that the histiocytes contained the test material itself (all high dosed females, 3 males and 1 male in the mid and low dose group, respectively). Positive staining for copovidone was also noted in the two recovery females of the highest dose group. The cytoplasm of the histiocytes contained either large clear vacuoles or showed a foamy appearance. No collateral damage was associated with the vacuolated histiocytes.

3. Discussion and Conclusion

Foamy histiocytosis was noted with a dose-response relationship after 90-day exposure of rats with CALCIUM LIGNOSULFONATE and slight regression after 28-day treatment-free period. This was not considered adverse effect in the light of regression and the absence of other lesion but to be the normal physiological reaction towards exposure to high molecular weight substance. Importantly, histiocyte infiltration or morphological changes of tissue macrophages were not noted in any other organ than mesenteric lymph nodes.

The observation of foamy histiocytosis in mesenteric lymph nodes is considered to be a common macromolecule related finding. Foamy histiocytosis was also noted in toxicity studies with other macromolecules e.g. copovidone (a copolymer of vinylpyrrolidone and vinyl acetate) or polypentosan sulfate sodium salt. For both substances, long-term toxicity studies demonstrate that (i) foamy histiocytes are present in mesenteric lymph nodes; however, without collateral damage and that (ii) progress to more severe findings like necrosis or inflammation is not to be expected. Neither PPS nor copovidone were carcinogenic in rats.

Administration of PPS in addition resulted in foamy macrophages in other organs like lungs or rectum, rectal inflammation, and liver cell vacuolization after 90-day treatment period. These latter findings, however, were not evident for CALCIUM LIGNOSULFONATE or copovidone after 90-day exposure. It is thought that these differences are attributed to low molecular weight of PPS and consequent differences in reaction of intestinal macrophages (Pittman et al., 1976; Weiner, 1988 and references therein).

Conclusion

The observation of foamy histiocytosis in the mesenteric lymph nodes in the 90-day feeding study with CALCIUM LIGNOSULFONATE is considered to be a normal physiological response to exposure with a macromolecule and does not represent an adverse response esp. in the absence of collateral damage like necrosis or inflammation and slight regression after cessation. Furthermore, it is considered that progress with time is rather unlikely. This conclusion is supported by toxicological data of other macromolecules like polypentosan sulfate sodium salt or copovidone.

It was therefore concluded that the NOAEL in the 90-day study with CALCIUM LIGNOSULFONATE is 2000 mg/kg bw/day.

4. References

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5. Appendix

Table 5-1 Histopathological changes observed in rats after administration of PPS in male rat

Males	13-week						2-year			
	0	63	125	250	500	1000	0	14	42	126
	mg/kg bw/day									
Rectum[a]	10	10	10	10	10	9	48	48	49	45
Myxomatous changes[b]							0	1(2.0)	3(1.3)	25(1.0)
Histiocyte infiltration	0	0	3(1.0)	7(1.1)	9(1.6)	8(2.0)	0	0	0	4(1.0)
Chronic inflammation	0	0	2(1.0)	7(1.1)	8(1.1)	8(1.8)	1(2.0)	0	1(3.0)	5(1.8)
Erosion							0	0	2(2.5)	2(2.0)
Ulcer	0	0	1(1.0)	3(1.0)	7(1.0)	7(1.4)	0	0	0	1(1.0)
Mandibular lymph node	10	1	10	10	10	10	5	5	9	2
Histiocyte infiltration	0	0	0	3(1.0)	8(1.1)	7(1.3)	0	0	0	0
Mesenteric lymph node	10	1	10	10	10	9	50	50	50	49
Histiocyte infiltration	0	0	0	6(1.0)	8(1.4)	8(1.8)	1(2.0)	1(2.0)	18(1.2)	39(1.5)
Lung	10	10	10	10	10	10	50	50	50	50
Histiocyte infiltration alveoli	0	2(1.0)	5(1.0)	6(1.2)	10(1.1)	8(1.3)	0	0	0	1
Chronic inflammation interstitial	0	0	0	0	1(1.0)	3(1.0)	0	0	0	0
Chronic inflammation alveolar							0	6(1.0)	11(1.4)	14(1.6)
Kidney	10	1	0	0	10	9	50	50	50	50
Renal tubular vacuolisation	0	0	--	--	0	8(1.0)	0	0	0	0
Liver	10	2	2	10	10	10	50	50	50	50
Vacuolisation cytoplasmatic	0	1(1.0)	0	0	4(1.0)	8(1.6)	0	0	0	0
Granulomatous inflammation	0	0	0	0	4(1.0)	6(1.2)	30	26	32	27
Spleen							50	50	50	50
Hyperplasia lymphohistiocytic							2(2.0)	2(2.0)	2(2.0)	8(2.8)

a number of animals examined
b number of animals with lesion (mean severity)

Table 5-2 Histopathological changes observed in rats after administration of PPS in female rat

Females	13-week						2-year			
	0	63	125	250	500	1000	0	28	84	252
	mg/kg bw/day									
Rectum[a]	10	10	10	10	10	10	46	43	44	42
Myxomatous changes[b]							0	1(1.0)	12(1.1)	35(1.1)
Histiocyte infiltration	0	2(1.0)	1(1.0)	2(1.0)	10(1.5)	10(2.0)	0	0	0	18(1.2)
Acute inflammation							0	0	0	1(2.0)
Chronic inflammation	0	0	0	0	9(1.0)	10(1.4)	0	0	1(2.0)	1(1.0)
Ulcer	0	0	0	0	6(1.0)	8(1.0)	0	0	0	0
Mandibular lymph node	10	1	2	10	10	10	3	8	3	2
Histiocyte infiltration	0	1(1.0)	2(1.0)	3(1.0)	3(1.0)	10(1.2)	0	1	0	0
Mesenteric lymph node	10	2	5	7	10	10	50	50	50	49
Histiocyte infiltration	0	2(1.0)	5(1.2)	7(1.0)	7(1.0)	10(1.1)	0	3(1.3)	27(1.3)	42(1.5)
Lung	10	10	10	10	10	10	50	50	50	50
Histiocyte infiltration alveoli	0	7(1.0)	5(1.0)	6(1.0)	7(1.0)	9(1.3)	0	0	1	0
Chronic inflammation interstitial	0	0	0	0	1(1.0)	3(1.0)	0	0	0	0
Chronic inflammation alveolar							2(1.0)	25(1.3)	27(1.6)	34(2.1)
Kidney	10	0	0	0	10	10	50	50	50	50
Renal tubular vacuolisation	0	--	--	--	0	10(1.0)	0	0	0	0
Liver	10	10	10	10	10	10	50	50	50	50
Vacuolisation cytoplasmatic	0	0	0	0	0	7(1.0)	0	0	0	0
Granulomatous inflammation	9(1.2)	10(1.0)	10(1.0)	10(1.0)	9(1.2)	10(1.3)	36	31	37	39
Spleen							50	50	50	50
Hyperplasia lymphohistiocytic							0	1(2.0)	2(2.5)	4(3.3)

a number of animals examined
b number of animals with lesion (mean severity)