

铬革渣资源化处理研究Ⅷ

——饲料胶原蛋白粉的毒理试验

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摘要 饲料胶原蛋白粉的 $LD_{50} > 10g/kg$, 属实际无毒级; 蓄积系数 $K > 5.28$, 属弱蓄积性; Ames 试验不论加 S-9 混合液与否, 致突变作用结果为阴性; 微核试验剂量高达 $5g/kg$ 仍为阴性; 精子畸形试验剂量高达 $5g/kg$ 亦呈阴性。

关键词 铬革渣, 胶原蛋白, 毒理试验。

制革过程中铬鞣后匀削、剪裁产生的废渣(铬革渣)量大面广, 对环境污染严重, 制革工业发达的国家都在致力于寻求有效的处理方法。铬革渣的主要成分是蛋白质, 将此蛋白质提取并加工成饲料胶原蛋白粉, 一方面可消除铬革渣对环境的污染, 另一方面又可为饲料工业提供一种新型的蛋白质资源。但由于从生皮到鞣革, 需贮存、运输、加工, 既会遭受病菌感染, 又要接触到多种化学物质, 尤其是铬鞣剂均由六价铬(重铬酸钠)还原为三价铬, 因此铬革渣中是否会有六价铬残留, 在从铬革渣中提取蛋白质并加工成饲料胶原蛋白粉的工艺过程中, 三价铬是否会有部分转化为六价铬, 以及三价铬在蛋白粉中的残留量, 蛋白质在加工过程中复杂的化学、物理变化是否会产生有毒有害物质, 总之, 从铬革渣中提取的蛋白质能否用作饲料蛋白质添加剂, 首先决定于其有否毒性。

由铬革渣制取饲料蛋白粉, 国内外都做了许多工作^[1-3], 但未见毒理学方面的报道。采用蒋挺大^[4]研究成的铬革渣资源化处理技术的两个工厂生产的饲料胶原蛋白粉, 分别在两个省级卫生防疫站按卫生部(85)卫防字第 78 号《食品安全性毒理学评价程序(试行)》进行毒理试验, 结果都一致, 表明饲料胶原蛋白粉无毒副作用。从喂养试验^[5]、营养学研究^[6]和产品大量投放市场 5 年来的实际使用情况调查, 采用本技术生产的饲料胶原蛋白粉是安全可靠的, 现将毒理试验结果报道如下。

1 材料与方法

1.1 饲料胶原蛋白粉

苏州市三马胶原蛋白厂产品, 含粗蛋白 58%, 水分 4.6%, 粗纤维 8.7%, 粗灰分 6.8%, 钙 1.02%, 磷 0.65%, $Cr(III)$ 16mg/kg (六价铬未检出)。

1.2 小鼠急性毒性试验

选 NIH 纯种健康小白鼠 40 只, 体重 18—22g, 雌雄各半。禁食 16h, 将受试小鼠按性别、体重随机分成 10.00、4.64、2.15 和 1.00g/kg 体重 4 个剂量组。饲料胶原蛋白粉样品以双蒸水配成所需剂量的灌胃液, 经口一次灌胃, 灌胃容量为 0.4ml/20g 鼠。观察 1 周, 记录小鼠的死亡情况, 以 Horn 法计算小鼠经口 LD_{50} 。

1.3 小鼠蓄积毒性试验

选 NIH 纯种健康小鼠雌雄各 20 只, 体重 18—22g。采用蓄积系数法进行试验。小鼠起始灌胃剂量为 1000mg/kg 体重, 即 0.1 LD_{50} , 以后每 4d 递增 1.5 倍, 并称体重调整灌胃量, 连续灌胃 20d, 逐天记录小鼠死亡数。

1.4 Ames 试验

(1) 菌株经鉴定, 基因型符合要求的鼠伤寒沙门氏菌 TA97a、TA98、TA100 及 TA102。各菌株过夜培养液细菌浓度均在 10^4 个/ml 或以上。

(2) S-9 由 Aroclor 1254 诱导的大鼠肝匀浆, S-9 混合液中 S-9 含量为 10%。

(3)剂量 饲料胶原蛋白粉研细过 140 目筛,以双蒸水配制成 4 个不同剂量的受试液(最高剂量的受试液有明显的沉淀)。阳性对照物 Dexon 和 α -氨基酚(α -AF)分别用双蒸水及二甲亚砜(DMSO)溶解。

(4)方法 平板掺入法,测试 2 次,每次每个剂量段平行样 3 个。

1.5 微核试验

25—30g 健康 NIH 纯种小鼠雌雄各 30 只。将小鼠按性别和体重随机分成 12 组,每组 5 只,雌雄鼠各设 4 个剂量组,一个溶剂对照组及一个阳性对照组、饲料胶原蛋白粉样品以双蒸水配成所需剂量的灌胃液,给动物灌胃 2 次,间隔 24h,第二次灌胃后 6h 杀鼠,取股骨骨髓悬于小牛血清中,直接涂片、固定、染色、镜检,记录具微核嗜多染红细胞数,以 2 项分布统计结果。

1.6 精子畸形试验

选 NIH 健康雄性小鼠 30 只,体重 25—35g。将动物按体重随机分入 4 个剂量组,一个阴性(溶剂)对照组和一个阳性对照组。饲料胶原蛋白粉样品以双蒸水配成所需剂量的灌胃液后,每天给小鼠灌胃 1 次,共 5d,末次灌胃后 30d 杀鼠,取样、制片、镜检,计算畸形率并用 Wilcoxon 秩和

检验法统计处理。

2 结果和讨论

2.1 急性毒性

灌胃后各剂量组小鼠未见异常,亦无死亡,雌雄小鼠经口 $LD_{50}>10g/kg$,按我国化学品急性毒性分级标准,该饲料胶原蛋白粉样品属实际无毒级。

2.2 蓄积毒性

雌雄小鼠于第 16d,累计剂量达 $3250mg/kg$,即 $3.25LD_{50}$ 时,各死亡 1 只,其余小鼠灌胃剂量累计达 $5275mg/kg$,即 $5.275LD_{50}$ 均存活,且体重普遍持续上升,雌雄小鼠蓄积系数 $K>5.275$,按蓄积评价标准,该饲料胶原蛋白粉样品属弱蓄积性。

2.3 Ames 试验

表 1 列出了饲料胶原蛋白粉的 Ames 试验结果。在本试验条件下,已知阳性对照结果为阳性。由表 1 可见,无论加 S-9 混合液与否,样品各剂量组与相应溶剂对照,均未见回变菌落数增多,且无剂量-反应关系,表明即使剂量高达 $5000\mu g/皿$ (有明显沉淀),该饲料胶原蛋白粉样品的致突变作用结果为阴性。

表 1 饲料胶原蛋白粉 Ames 试验¹⁾

组 别	剂量 ($\mu\text{g}/\text{皿}$)	TA98		TA97a		TA100		TA102	
		-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9
溶剂对照									
双蒸水	0.1 ml/皿	41±2	44±4	144±7	169±13	159±12	177±8	239±13	322±17
DMSO	0.1ml/皿	38±4	40±6	138±11	158±15	151±20	164±17	210±21	309±24
样品									
蛋白粉	40	41±3	45±3	143± 5	164±11	166± 7	182± 6	240±22	320±18
	200	40±3	44±3	139±10	167±10	160± 7	182±12	232±18	312±22
	1000	42±2	46±3	144± 1	163±16	166±11	174±17	238±13	316±21
	5000	41±2	45±6	143±10	166± 9	163±13	181±8	235±16	354±33
阳性对照									
Dexon	50	2170±288		2072±509		1915±536		3219±493	
α -AF	10	1591±667		4061±1041		2592±272			

1)表内数据为均数 $\pm$ 标准差

2.4 微核试验

各实验组小鼠的具微核嗜多染红细胞数及其千分率列于表 2。由表 2 可见,阳性对照组具

微核嗜多染红细胞数与溶剂对照组比较,差异非常显著($P<0.01$),说明本实验方法是可靠的。样品的各剂量组小鼠具微核嗜多染红细胞率与

溶剂对照值之间均无显著性差异($P>0.05$),且 蛋白粉样品仍未表现出对小鼠骨髓细胞染色体无剂量-反应关系,剂量高达 5g/kg 时饲料胶原 断裂效应及纺锤体毒效应,即试验结果为阴性。

表 2 饲料胶原蛋白粉的微核试验

组 别	剂量(g/kg)	性别	鼠数	观察细胞数	微核细胞数	微核细胞率(%)	P
溶剂对照							
双蒸水	0. 2ml/10g	雌	5	5000	6	1. 2±0. 8	
		雄	5	5000	7	1. 4±1. 1	
样品							
蛋白粉	0. 62	雌	5	5000	6	1. 2±0. 8	>0. 05
		雄	5	5000	6	1. 2±0. 8	>0. 05
	1. 25	雌	5	5000	5	1. 0±0. 1	>0. 05
		雄	5	5000	6	1. 2±0. 4	>0. 05
	2. 50	雌	5	5000	6	1. 2±0. 4	>0. 05
		雄	5	5000	6	1. 2±0. 8	>0. 05
	5. 00	雌	5	5000	7	1. 4±1. 1	>0. 05
		雄	5	5000	5	1. 0±0. 7	>0. 05
阳性对照							
环磷酸胺	0. 03	雌	5	5000	109	21. 8±1. 9	<0. 01
		雄	5	5000	117	23. 4±1. 9	<0. 01

表 3 饲料胶原蛋白粉对精子畸形率的影响

组 别	剂量(mg/kg)	鼠 数	观察精子数	畸形精子数	畸形率(‰)	P
阴性对照						
双蒸水	20ml/kg	5	5000	71	14.2	
样 品 蛋白粉	625	5	5000	74	14.8	>0.05
	1250	5	5000	76	15.2	>0.05
	2500	5	5000	75	15.0	>0.05
	5000	5	5000	77	15.4	>0.05
阳性对照						
用磷酸甲酯 (慢性)大鼠	100	5	5000	362	72.4	<0.01

2.5 小鼠精子畸形试验

饲料胶原蛋白粉对小鼠精子畸形率的影响见表 3。阳性对照组与阴性对照组的精子畸形率比较有非常显著意义($P<0.01$),说明本实验方法是可靠的。样品各剂量组的精子畸形率与阳性对照值相比,均无显著性差异($P>0.05$),且无剂量-反应关系,表明剂量高达 5g/kg 时仍显示饲料胶原蛋白粉样品对小鼠精子畸形试验为阴性。

由以上毒理试验结果可见,采用铬渣资源化技术生产的饲料胶原蛋白粉用于饲料喂

养禽畜是安全可靠的。

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Abstracts

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absorption spectrometry, plasma mass spectrometry, X-ray fluorescence, neutron activation, etc. For each of most elements, two or more different, reliable analytic methods were adopted. A computer was used to process the data obtained by statistical method. Any group-outliers were rejected by using Grubbs, T, Dixon, Multi-level comparative S method, average geometric variation-Grubbs test. The best estimated values were obtained by taking the averages of preferred averages of all the data and analytic data as the four central values. The best estimated values thus calculated and complied with the certified values rule are taken as standard values. An evaluation of stability of the samples was made by X-ray fluorescence spectrometry during one year period. The results indicated that the storage stability of this standard reference material was satisfactory.

Key words: environmental soils standard reference material, stability, minimal amount of sampling, comparison of precision, certified values analysis, four central values.

The Optimisation of Grey non-linear River water Pollution Control System Using A Two Level Method. Zhang Xiangwei (Water Quality Research Center of China Beijing 100044); *Chin. J. Environ. Sci.*, 15(1), 1994, pp. 25—30

This paper focuses on the water quality planning problem of grey non-linear river water pollution control system using ideas of the grey system theory. Grey non-linear model and a two level method have been developed, which not only can describe the imperfection of water quality planning information but also can provide a new approach of dealing with the higher order, higher dimension and non-linear water quality planning model. The major studies involved are of three aspects: (1) Grey convex set, grey convex function and grey convex programme are defined; (2) Kuhn-Tucker condition for grey non-linear planning model has been given and (3) the optimisation of grey non-linear river water pollution control planning model using a two level method.

Key words: grey system theory, water pollution control system, two level method.

Sediment Oxygen Demand in the Yuancun Reach of the Pearl River in Guangzhou. Liu Fuqiang, Qi Sang (Institute of Aquatic Ecoscience, Jinan University, Guangzhou 510632); *Chin. J. Environ. Sci.*, 15(1), 1994, pp. 31—35

The physical properties, characteristics of oxygen demand, proportions of chemical oxidation and biological respiration in the total oxygen demand of

sediments from the Yuancun Reach of the Pearl River in Guangzhou, were studied in laboratory during mean water and high water seasons. Results indicate that the sediment only consumed oxygen initially over the first six hours in mean water season, while in high water season, the sediment continuously consumed oxygen at a comparatively low rate. It is doubt less that chemical oxygen demand plays a main role in total oxygen demand in both hydrological seasons. At the same temperature and water flow rate, the rate of sediment oxygen uptake in high water season is significantly higher than that in mean water season. In addition, the rates of sediment oxygen demand are related to the temperature and flow rate and their equations are: $SOD_M = 0.4945 \times 1.0058^{T-20}$, $SOD_H = 0.6155 \times 1.0234^{T-20}$, $SOD_M = 0.1623 \times 1.0912$, $SOD_H = 0.2393 \times 1.0857$.

Key words: Pearl River, sediment, biological oxygen demand, non-biological oxygen demand.

Study on the Treatment of Chrome-Leather Scraps as a Resource; VIII Toxicological Test of Feed Collagen Protein. Jiang Tingda, Zhang Chunping (Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085); *Chin. J. Environ. Sci.*, 15(1), 1994, pp. 36—38

The feed collage protein powder has a $LD_{50} > 10g/kg$, and can be assessed to be in an actually nontoxic grade. It has an accumulative coefficient $K > 5.28$, which is considered to be weakly accumulative. The Ames test result of mutagenesis is negative, either with or without adding S-9 mixed liquid. The micronucleus test and spermatozoon malformation test also showed a negative reaction when dosage exceeded 5g/kg.

Key words: feed collage protein, toxicology.

Kitchen Wastewater Treatment by Iron-Carbon Flocculating Bed. He Weiguang, Guan Yaochu et al. (Chemistry Department of Zhongshan University); *Chin. J. Environ. Sci.*, 15(1), 1994, pp. 39—41

Kitchen wastewater can be treated by iron-carbon flocculating bed equipment. It was found that this method can be used to remove the animal and vegetable oils, COD, and BOD from kitchen wastewater with high removal rates, for example, of 96%, 72.5% and 90%, respectively.

Key words: iron-carbon flocculating bed, kitchen wastewater.

The Adsorption-Flocculation Method Using Bentonite for Treatment of Organic Dye-Containing Wastewater. Hang Hu, Hu Bolu et al. (Dept. of