

**Amendment 1 to REPORT No. 2500147
Regulatory Document**

DSM 

Date:	17-June-2008
Author:	Beck M
Report Title:	Absorption, Distribution and Excretion of Tritium Labelled Lignosulfonate after Single Oral Administration to Rats
Amendment No	1
Project No.	6309
Compound	Ca-Lignosulfonate, Food Grade

Justification for Amendment

To specify the use of the word 'radiolysis' in the document.

Clarification



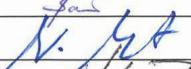
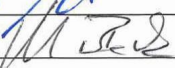
In several passages within the Regulatory Document Report 2500147, the expression *radiolysis* was used, which might be unclear or misleading. '*Radiolysis*' is mentioned in the introduction (page 2), in the chapters of the preparation of the tritium-labeled stock solution of the test item (page 5), experimental procedures (page 7), and in the results and discussion chapter (pages 10, 11, 14, 15, 17)

In the following paragraph it is explained and commented what was meant.

Distribution
Karin Feltes, NBD/RH
RDR System

NRD/CH - Neupert W
NRD/CH Safety Archive

Approved

<u>Name</u>	<u>Signature</u>	<u>Date</u>
Main Author Mareike Beck		17.6.08
Principal Scientist / Competence Mgr Jochen Bausch		17 June 08
Research Center Head Werner Neupert		18/06/08
Project Manager Markus Beck		23 Jun 08

The predominant risk associated with using ^3H -labeled compounds in biological systems is the potential chemical and metabolic instability of the tritium atom itself at its specific site within the test molecule. In aqueous physiological environments of varying pH, tritiated water (HTO) is readily and unavoidably formed by hydrogen exchange processes. This is a well known phenomenon when using tritiated compounds in ADME studies.

Tritiated water formed in the aqueous application solution used in the lignosulfonate study was separated as much as possible by several ultracentrifugation steps before administration to the animals. After administration, the tritium-hydrogen exchange continuous in the gastrointestinal tract of treated animals. In case of compounds with very low absorption, such as lignosulfonate, ^3H -radioactivity found in tissues, blood and urine of test animals is extremely misleading, as HTO is readily absorbed, widely spread within the body, and in most cases of longer persistence than the compound of interest. To overcome this problem, quantification of HTO within all samples via lyophilization is sought, as was done in our study with lignosulfonate.

Radiolysis is the dissociation of molecules by radiation. This is a process known to occur with many radioisotopically labelled compounds over time (also ^{14}C). Tritium-hydrogen exchange could be seen as one form of radiolysis. For our ADME studies with ^3H -lignosulfonate, we cannot exclude formation of other small MW components by radiolysis. However, we have shown that the by far major part of the radioactivity found in tissues and liquid samples can be attributed to tritiated water.