

creasorb

Toxicological and Environmental Safety Data

STOCKOSORB®

Introduction

STOCKOSORB® is a crosslinked copolymer of acrylic acid and acrylamide partially neutralized as potassium/ammonium salt.

The polymer is in its dry form a granulate and forms a gel-like material upon addition of water or watery solutions. Due to the crosslinking it is insoluble in water.

Uptake of water is facilitated mainly by the negative carboxylic groups of the polymer and their hydration with water molecules. Complete solubilization is hindered because of the crosslinking of different polymer chains. Due to incomplete crosslinking small amounts (generally about 6 %) can be leached out of the polymer matrix by water, the so-called water extracts. To these the environment can be potentially exposed to besides the gel.

Specific analytical means for determination of STOCKOSORB® in a complex environmental matrix are not available besides of ¹⁴C-radiolabelling in laboratory experiments.

Depending on the method, determination of the polymer and its water extracts in particular may be quantified by the total organic carbon (TOC) parameter, later on referred to as "mg carbon/l". The water extracts comprise low (oligomers) to high molar mass molecules of up to approximately



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2 x 10⁶ dalton in molar mass. Besides this the polymer matrix still contains a small amount of residual acrylamide and acrylic acid all of which is in the salt form with potassium.

The following data have been generated for the superabsorbent polymer STOCKOSORB®. The testing was performed after relevant exposure scenarios were identified and the necessary tests derived from intended and reasonably anticipated exposure situations.

However, it should be taken into consideration that the data to follow were generated under laboratory conditions and have to be extrapolated to environmental conditions. Therefore, caution should be exercised in applying the generated figures for accurate estimation of actual environmental risks.

The presented toxicological and ecotoxicological studies were performed according to international recognized test methods and in compliance with the "Principles of Good Laboratory Practice (GLP)". Note: Not all of the different variations of the superabsorbent polymers of the STOCKOSORB®-series were tested with all of the following test systems due to ethical and economical reasons. Some data were drawn in analogy.

TOXICITY DATA

Acute Oral Toxicity (Limit-Test)

Approximate (A) LD50 > 5000 mg/kg body weight of up to 3 % (w/v) STOCKOSORB® as a gel in 0.9 % (w/v) saline applied with stomach tube to 5 male and 5 female rats each. No toxic symptoms could be observed; body weight development was normal during observation for 14 days after application; necropsy revealed no visible organ alterations, i.e. the test substance has to be regarded as essentially non-toxic after oral intake.

Acute Dermal Toxicity (Limit-Test)

Approximate (A) LD50 > 2000 mg/kg body weight of up to 3 % (w/v) of STOCKOSORB® as a gel in a 0.9 % (w/v) saline applied to the shorn skin of 5 male and 5 female rats. No toxic symptoms; body weight development was normal for 14 days after application; necropsy revealed no visible organ alterations; no deaths occurred, i.e. STOCKOSORB® is essentially non-toxic when applied dermally.

Subacute Skin Compatibility

Repeated application of a 3 % (w/v) gel in 0.9 % (w/v) saline of STOCKOSORB® onto the shorn skin, without occlusion, of 10 female mice, revealed occasionally, very slight transient edemas accompanied with a slight increase in skinfold thickness. The test substance was applied 3 times per week over 8 weeks. Body weight was within normal range; no systemic effects, due to the application of the test substance, were observed, i.e. the test substance, in its gelled state, shows good skin compatibility.

Acute Eye Irritation

Application of 0.1 g of STOCKOSORB® into the conjunctival sac of the eyes of rabbits caused slight erythema of eyes and adjacent mucous membranes. Further lesions were not observed. There were no systemic symptoms due to the application of the test substance.

The observed slight irritative effect onto mucous membranes of the eyes are caused by the somewhat abrasive properties of the dry, crystalline granulate of STOCKOSORB® and its capacity to dry out the membranes due to the uptake of fluid. The watery suspension of STOCKOSORB® exerts no such effects on the eyes of rabbits.

Hens' Egg Test

The Hen's Egg Test is an alternative test method to reduce animal testing. With this method irritative substance effects on membranes such as mucus and eye can be determined. Chorion allantois membrane (CAM) testing in hen's eggs revealed only moderate irritative effects when 200 mg of the dry granulate of STOCKOSORB® were used. Application of a 3 % (w/v) gel led to no effects on the CAM. The observed effects are due to the drying-out properties of the granulate test substance and are therefore characterized as physical, not chemical effects.

Cell Toxicity

STOCKOSORB® was examined regarding its influence on mammalian cells in a cell culture system using a fibroblastic cell line derived from mice. The cells were incubated for 24 hours with an extract of STOCKOSORB® (15 g/l of medium) in concentrations up to 100 %. No adverse effects on the morphology or viability of the cells were observed.

Skin Sensitization

STOCKOSORB® was tested with respect to its potential to sensitize animals after skin contact according to the maximization test of Magnusson and Kligman. Ten (10) male and ten (10) female guinea pigs were treated intradermally with a water extract [1.5 % (w/v)] and dermally with a 3.0 % (w/v) gel in 0.9 % (w/v) saline during the induction period.

The challenge was executed with a 0.3 % (w/v) gel in 0.9 % (w/v) saline. No erythemas or edemas were observed, therefore, it is unlikely for STOCKOSORB® to exhibit a potential for skin sensitization.

Elimination following oral administration

A study was performed to provide information on the absorption, excretion and plasma kinetics of total radioactivity following single oral administration of ¹⁴C-STOCKOSORB® to rats.

Excretion of radioactivity was rapid with nearly all of the dose recovered within 48 h post dose. Faeces was the major route of excretion accounting for more than 90 % of the applied dose. A further 3 % of the dose was excreted via urine. Negligible amounts of radioactivity were retained in the carcass after 5 days. The presence of only very low concentrations of radioactivity in the plasma indicates as well, that the oral dose is poorly absorbed and the small amount of radioactivity which was absorbed was eliminated quickly.

Mutagenicity Test with Bacteria

For testing mutagenicity an Ames Test was executed with an extract of STOCKOSORB® [20 g/l in 0.9 % (w/v) saline with 10 % (v/v) ethanol] with and without metabolic activation by rat liver microsomes. Extracts of STOCKOSORB® were tested in 4 different strains of histidin requiring *Salmonella typhimurium* (base pair substitution and frame shift mutation) and

2 tryptophan requiring strains of *Escheria coli* for their ability to induce point mutations with and without the presence of a metabolic activation system. Up to 5000 micrograms/plate no mutagenic events could be observed. Furthermore, cytotoxicity was not detected up to 5000 micrograms/plate.

Therefore, there was no indication of a mutagenic potential to bacteria of the extract of STOCKOSORB® up to the equivalent of 5000 micrograms/plate.

Mutagenicity Test with Mammalian Cells

Mouse Lymphoma L 5178 Y Cells were exposed with

(S 9 mix from Aroclor induced rat hepatocytes) and without metabolic activation to an extract of STOCKOSORB® [20 g/l in 0.9 % (w/v) saline with 10 % (v/v) ethanol]. The test substance failed to induce point mutations at the HPRT locus up to the equivalent of 400 micrograms/ml.

Conclusions regarding Toxicology

STOCKOSORB® is devoid of any potential adverse effects provided direct contact to mucous membranes is avoided (by protective cloth and goggles). Usage of STOCKOSORB® has no negative effects on the health of users due to the low toxicity profile.

ECOTOXICITY DATA

Toxicity to Bacteria

Growth behavior and propagation of the microorganism *Pseudomonas putida* was determined with a saline (0.9 %) extract of STOCKOSORB®. Negative effects onto the growth behavior of the *Pseudomonas putida* were not observed, i.e. no cytotoxic, cytostatic or biocidal effects are to be expected. The EC50-value for half maximum propagation is higher than the highest concentration tested, i.e. 1768 mg carbon/l which equals 8 g polymer/l. Therefore adverse effects on bacteria are not expected when relevant environmental exposure is considered.

Toxicity to Algae

Growth behavior of single cellular algae *Scenedesmus subspicatus* was determined with a soluble extract of STOCKOSORB® up to 910 mg carbon/l (5 g STOCKOSORB®/l). From 20 mg carbon/l inhibition of growth was observed. The EC50-value which defines half maximum growth was 150 mg carbon/l which is the equivalent of 1 g polymer/l. Higher concentration led to further growth reduc-

tion. The observed slight to moderate toxicity is thought to be of no practical importance, when realistic environmental exposure conditions are taken into consideration.

Toxicity to Ciliates

Cells of the ciliate *Tetrahymena pyriformis* were incubated for 48 hours with the watery extract of STOCKOSORB® in concentration up to 1000 mg carbon/l (corresponding 6 g/l polymer). No negative effects on growth behavior were observed. The EC50-value for half maximum cell propagation was greater than the highest concentration tested. Therefore biocidal effects are not expected under relevant environmental exposure conditions.

Toxicity to Waterpolyps

Cytotoxic effects and inhibition of reproduction of the waterpolyp *Hydra littoralis* were determined with a watery extract of STOCKOSORB® with concentrations up to 400 mg carbon/l (5 g polymer/l). At low concentrations no cytotoxic effects were observed and the reproduction rate was increased in comparison to the control. Higher concentrations led to cytotoxic symptoms and a reduction on reproduction. The EC50-value defined as the concentration of the test substance which restricts the reproduction rate by 50 % is approximately 140 mg carbon/l which equals 1.6 g polymer/l. Therefore adverse effects on waterpolyps are not expected under realistic environmental exposure conditions.

Toxicity to Daphnids

Acute effects of STOCKOSORB® on the swimming ability of the daphnids *Daphnia magna* for a 48 hours period was determined with a soluble extract of STOCKOSORB® at concentrations of up to 1600 mg carbon/l which is the equivalent of 10 g STOCKOSORB®/l. Up to 400 mg carbon/l no toxicity effects on the daphnids could be observed. The EC50-value for half maximum inability to swim is approximately 980 mg carbon/l which equals 6 g STOCKOSORB®/l. Therefore, under appropriate use

conditions, no critical deleterious effects are expected on daphnids.

Acute Toxicity to Fish

To determine acute lethal effects to fish the cold water species *Leuciscus idus* (golden orf) and the warm water species *Brachydanio rerio* (zebra fish) were exposed to watery extracts of STOCKOSORB®. The LC50-value which defines the mean lethal concentration is approximately 250 mg carbon/l which equals 3.6 g polymer/l for golden orf and higher than 300 mg carbon/l which equals 5 g polymer/l for zebra fish.

Chronic Toxicity to Fish

To determine chronic toxic effects the fish *Brachydanio rerio* (zebra fish) was exposed to watery extracts of STOCKOSORB® over a time period of 10 days. No deaths were observed at the highest concentration tested and the no-observable-effect-level for the prolonged toxicity test with zebra fish is higher than 300 mg carbon/l which equals 5 g polymer/l. Therefore STOCKOSORB® has to be regarded as practically non-toxic to fish.

Toxicity to Birds

Acute toxicity of STOCKOSORB® to the bird *Colinus virginianus* (bob white quail) was determined by oral application. As no deaths or adverse clinical effects were observed at a dose level of 2000 mg/kg the LD50-value for half maximum lethality is greater than 2000 mg/kg. Therefore no critical effects to birds are expected under relevant exposure conditions.

Toxicity to Earthworms

To determine acute toxic effects to earthworms *Eisenia foetida* was exposed to STOCKOSORB® over a 14 day exposure period. The test soil was prepared at a limit concentration of 20 g STOCKOSORB®/kg soil. As no unusual behavior was noted throughout the study period and the cumulative

percentage mortality was similar as in the control vessels the LC50-value for half maximum lethality is greater than 20 g/kg.

Therefore adverse effects on earthworms are not expected under appropriate exposure conditions.

Cress Germination Test

The influence of STOCKOSORB® on garden cress (*Lepidium sativum*) was determined with water-soluble extracts of STOCKOSORB® up to a concentration of 993 mg carbon/l (7 g STOCKOSORB®/l). After 5 days development status and root length of plants treated with STOCKOSORB® were compared with plants without STOCKOSORB®. In concentrations of up to 3 g/l STOCKOSORB® had a positive influence on root growth and yielded a higher dry weight of the plants.

Higher concentrations had a slight negative effect. Seedlings growth with a 1 % STOCKOSORB® gel were in good condition in general, with some decrease in root length vs. control plants, but with increases in additional roots. The EC50-value of 900 mg carbon/l (6 g/l polymer) and the positive results in germination in the gel yield no expectation of critical effects under relevant conditions of use.

Terrestrial Plants Growth Test

Phytotoxic effects of STOCKOSORB® onto emergence and growth of seedlings over a 20 day period were determined with application rates of 0, 5, 10 and 20 g/kg soil of the following plants:

Phaseolus aureus (mung bean),
Triticum aestivum (wheat),
Lactuca sativa (lettuce),
Lepidium sativum (cress),
Lycopersicon esculentum (tomato) and
Cucumis sativus (cucumber).

Uptake by Plants

The uptake and translocation of STOCKOSORB® in plants has been investigated in wheat, carrots and lettuce. STOCKOSORB® was incorporated into sandy

loam topsoil at a target rate of 10 g/kg and the plants were grown from germination to maturity.

Plants were harvested at intervals between sowing and maturity and analyzed. The percentage of STOCKOSORB® taken up into the plants was very low at all time points with the highest level in lettuce at maturity accounting for only 0.12 % of the applied radioactivity. Re-fixation of ¹⁴CO₂ liberated by degradation of the polymer by soil microorganisms was not detected.

In order to clarify, if the radioactivity taken up comprises monomeric Acrylamide the lettuce plants were analyzed for their content of acrylamide additionally. No acrylamide was detected with a limit of quantification of 0.5 µg/kg.

To investigate the uptake of acrylamide in food crops several field trials with tomatoes, mushrooms, melons, and maize were performed. The plants and fungi were grown to maturity on soil or substrate amended with STOCKOSORB® under standard growth conditions. Following harvest the mature fruits and crops were analyzed for their content of acrylamide. In none of the samples acrylamide was detected with a limit of quantification of 0.5 µg/kg.

Conclusions regarding Ecotoxicity

STOCKOSORB® exhibits no relevant ecotoxicity when realistic environmental exposure conditions are taken into account. The use of STOCKOSORB® is safe for the environment.

Environmental Fate

Ready Biodegradability

To determine the ready biodegradability of STOCKOSORB® in a Modified-Sturm-Test (CO₂ evolution test) the water-soluble extract was exposed to activated sludge microorganisms at concentrations of 10 and 20 mg/l of dissolved organic carbon. The cumulative carbon dioxide release over a 28 day exposure period was used to calculate the percent-

tages of biodegradation. As no significant levels of biodegradation were recorded STOCKOSORB® is not readily biodegradable under aqueous, aerobic conditions.

Biodegradation in compost

Biodegradability of STOCKOSORB® was tested under solid, aerobic conditions in a controlled composting test. The inoculum consisting of stabilized and mature compost was mixed with 10 % (w/w) STOCKOSORB® and incubated for 45 days at temperatures following a real composting temperature profile. Biodegradation was calculated by the percentage of solid carbon of the test compound which has been converted to carbon dioxide. As after two weeks of incubation the final conversion level of 3 % was reached STOCKOSORB® has to be regarded as poorly biodegradable under aerobic conditions in compost.

Degradation and conversion processes during composting

The fate regarding degradation and conversion of a linear copolymer of acrylic acid and acrylamide was determined in a composting study. The polymer - not crosslinked and thus soluble - represents the water soluble part of STOCKOSORB®.

After the composting of the radiolabeled polymer for 45 days in a laboratory composting equipment the biodegradation rate (mineralisation) was calculated from the amount of carbon dioxide produced. Further the compost was extracted with water and sodium hydroxide solution to determine the distribution characteristic of the polymer in the water soluble part and in the soluble and insoluble humus fractions of the compost. Composting resulted in a biodegradation rate of 2.4 %. The remaining water soluble part was 26 % and further 5.3 % were found in the soluble fraction of the humic acids. However 63 % of the applied radioactivity were detected in the insoluble sediment indicating a high binding rate of the polymer to the humus fraction. Unspecific binding of the polymer to the

humus is excluded. The results indicate that the soluble part of STOCKOSORB® will be mineralised only partly during composting but the main fraction will become part of the humus matrix.

Biodegradation by fungi

The degradation of STOCKOSORB® by white-rot fungi, ubiquitous organisms which are capable to degrade the most recalcitrant biopolymer, lignin, was investigated. Liquid cultures of *Phanerochaete chrysosporium* were incubated with radiolabelled STOCKOSORB® as a gel under nutrient limited conditions and the rate of mineralization was monitored by quantitating the amount of $^{14}\text{CO}_2$ produced. The insoluble polymer was depolymerized within 3 weeks to water-soluble products by an extra-cellular enzymatic degradation system. The polymer degradation products were neither toxic to the fungus nor to other organisms as shown in toxicological tests with bacteria, algae, ciliates and daphnia. Over longer time periods up to 80 % of the water-soluble meta-bolites were incorporated in the fungal mycelial mat. Mineralization by the fungus occurred throughout the time course and although the overall amount of polymer mineralized was low, the results suggest that almost all of the polymer was degraded to fungal metabolites. The results have demonstrated that white-rot fungi are in principle capable of degrading STOCKOSORB®.

Biodegradation in soil by bacteria

To investigate the biodegradation by soil bacteria radiolabelled STOCKOSORB® was incubated with intact soil for 2 years. The rate of mineralization was monitored by quantitating the amount of $^{14}\text{CO}_2$ produced and the amount of radioactivity extractable as well as non-extractable from the soil was determined. Within the first few days a small increase in the CO_2 -evolution and a decrease in the extractable fraction was determined. The rate of mineralization was low throughout the time course with a slow but steady increase, and the cumulative

mineralization rate after 2 years was 3 %. The amount of non-extractable radioactivity was high during the whole time period indicating a high adsorption capacity of STOCKOSORB® to soil. The results confirm that soil bacteria are in principle capable of degrading STOCKOSORB® as long as certain molar masses are not exceeded; this is valid for the smaller oligomers and monomers which account for approximately 3 % of the water-soluble fraction.

Biodegradation in soil by bacteria and fungi

The synergistic effect of soil bacteria and white-rot fungi on the biodegradation of STOCKOSORB® in soil was investigated. The soil microcosms were prepared by mixing soil with radiolabelled STOCKOSORB® and with sawdust inoculated with the fungus *Phanerochaete chrysosporium*. Control microcosms with either steril soil but with fungus or with intact soil but uninoculated sawdust were prepared as well. Microcosms were maintained at 37 °C for 76 days and mineralization rates were determined by the amount of ¹⁴C₂O₂ produced. Solubilization of the polymer was estimated by the degree to which radioactivity distributed throughout the soil. While mineralization was minimal in microcosms with soil bacteria but without the fungus (0.35 %) significant mineralization (4.3 %) was determined in microcosms containing fungus but no bacteria. The highest mineralization was observed in microcosms containing soil bacteria and fungi accounting for 7.3 % in 76 days. STOCKOSORB® was solubilized in fungal-containing soil microcosms within 19 days demonstrated by monitoring the spread of radioactivity throughout the soil. Moreover, the amount of radioactivity observed was evenly spread throughout the microcosm within 76 days, suggesting that the polymer had been completely solubilized and spread within the fungal hyphae network, as it is incorporated by the fungus.

Biodegradation of ¹⁴C-STOCKOSORB® was also studied in agricultural soil by two white rot fungi (*Pleurotus ostreatus*, *Dichomitus squalens*), a brown rot fungus (*Flammulina velutipes*) and a saprophytic soil fungus (*Agaricus bitorquis*) using microcosms. The highest mineralisation of the copolymer to ¹⁴C₂O₂ was measured with *Pleurotus ostreatus* (8.8 % within 22 weeks in soil and 31 % within 28 weeks in a pure culture on wheat straw). This species also increased the portion of soluble components of STOCKOSORB® in soil significantly. The results indicate that fungi initiate significant conversion and degradation processes, leading to an increase of the polymer fraction which can be mineralized by soil microorganisms. The synergistically cooperation of white-rot fungi and soil bacteria will lead to the degradation of STOCKOSORB® with time.

Distribution in Terrestrial Systems

To investigate the adsorption and desorption characteristics of STOCKOSORB® the aqueous extractable components of radiolabelled ¹⁴C-STOCKOSORB® were incubated with four different soil types: sand, sandy loam, silty clay loam and clay loam. Significant adsorption of radioactivity resulted in all soil types following 16 h equilibration and correlated well with the soil organic matter content. In sand the lowest and in clay loam the highest adsorption was obtained.

Subsequent desorption was incomplete in all soil types. The magnitude of the Freundlich adsorption coefficient *K_f* indicates that STOCKOSORB® has a high capacity to adsorb to soil.

To determine the soil leaching potential STOCKOSORB® was applied as moistened gel to the surface of soil columns of each of the four soil types and leached for 5 days.

Migration throughout the columns was very low and leaching was in the order sand > sandy loam > silty clay loam > clay loam. The distribution coefficient for each of the soil types indicates that the potential for leaching is very low.

Heavy Metal Mobilization

A watery suspension of sludge and compost was incubated with STOCKOSORB® (0.1 % and 1 %) for 24 hours to investigate the heavy metal mobilization potential of STOCKOSORB®. After processing the concentrations of Zn, Cd, Pb, Ni, Fe, Mn, Cr, Cu, Mg, Al and P in the supernatant have been similar in comparison to water treated controls. Therefore it is concluded that STOCKOSORB® has no relevant potential to interact and mobilize heavy metal ions from compost or soil.

Conclusions regarding Environmental Fate

Taking into account all investigations with respect to biodegradability research has shown that STOCKOSORB® does not constitute a persistent polymer, but is susceptible to natural, fungal degradative processes known to occur in the environment.

After solubilization, degradation and mineralisation the constituents of the polymer are integrated into the natural carbon and nitrogen cycle. STOCKOSORB® is safe and compatible to the environment without any negative effect on the natural soil compartment.

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