# A New Antifungal Agent for the Leather Industry: S-Hexyl-S'-Chloromethyl-cyanodithiocarbimate (CHED)

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## INTRODUCTION

Raw hides are a rich source of fats and proteins that may serve as metabolic substrates for micro-organisms, especially bacteria and fungi. These micro-organisms that are capable of invading the hide for its nutrients are present on the fresh hide during harvest as well as from the surrounding environment during and after processing at the tannery. Fungal attack on tanned collagen changes the chemical composition of the pelt in the area of growth. These changes along with fungal pigmentation will usually cause discolouration or spots of various colours depending on the infesting organism. This fungal discolouration is difficult to correct and can spoil crust or finished leather. To protect tanned hides (such as wet-blues, vegetable-tanned, free-of-chrome, or oil-tanned leathers), a fungicide is added during the pickle or tanning process. The fungicide must survive the conditions of tannage and adhere to the surface of the tanned hide to protect the leather from the time the tanning process is complete until the tanned hide is processed further and finished into a dry leather product. In order to be effective for the leather industry, the fungicide should be stable under acid conditions, reasonably stable to UV-light, relatively unreactive with other tanning process chemicals, and have a high affinity for the hide or skin substrate.

Historically the leather industry made use of chemistries such as organomercurials and chlorophenates which are now banned because of high level dangers regarding environment and human exposure. In recent decades these have been replaced with effective and safer chemistries such as: 2-(thiocyanomethylthio)benzothiazole (TCMTB), 3-iodopropynylbutylcarbamate (IPBC), n-octyl-isothiazolinone (OIT), diiodomethyl-p-tolylsulfone (DIMTS), and some phenolics.

A need exists in the leather industry for new compounds that not only are effective fungicides, but also survive the chemical environment of the tanning process. In addition, a valuable strategy for microorganism control is to combine two or more microbicides to expand the spectrum of control as well as to exploit potential synergies in the biocidal actions. This paper describes chemical, biological, and toxicological properties of a new antifungal compound in a class of chemistry known as cyanodithiocarbimates. A patent for the use of this chemistry as a fungicide in the leather industry has been granted.[2]

## MATERIALS AND METHODS

### Chemistry

Buckman worked with cyanodithiocarbimates in the late 1960s. Various substitutions were evaluated and found to have good efficacy against fungi but at that time, the patented TCMTB was considered to be more effective as a fungicide and less expensive to manufacture. While exploring potential new fungicides, CHED and other alkyl, aromatic and heterocyclic substituted cyanodithiocarbimates, were used as intermediates. Magnetic resonance studies on the compounds to compare structure with efficacy showed that the greatest activity against fungi was evidenced in samples with the highest percentage of chloromethyl (bromomethyl) impurities remaining of the synthetic intermediate. When the purified chloromethyl intermediates were screened, they maintained the highest efficacy. There were literature precedents for use of carbimates against fungi and to a lesser degree against bacteria, in agricultural, wood treatment and latex paint applications, though preservation of leather was not investigated.[3,4]

The range of moieties substituted in the asymmetric substituted-chloromethyldithiocarbimates, was greatly expanded including straight chain and branched alkyl, alkenyl, alkynyl, aryl derivatives and some with halogen or alkyloxy components. The hexyl derivative, known as CHED, showed the greatest efficacy in leather. CHED has proven to be an effective antifungal preservative for leather as a single active substance or in combination with other fungicides. In comparison trials, performance is measured by preserving wet-blues for eight to ten weeks in a standard tropical chamber challenge test environment. Production trials of a formulation containing CHED, TCMTB and OIT have proven more cost effective than the industry benchmark 30% TCMTB (Busan 30L) in initial field trials. Other combinations are currently under investigation.

Initial testing has shown CHED to be at least as environmentally friendly as existing active substances used by the leather industry, if not more so. CHED will degrade by hydrolysis and the byproducts of CHED show very low toxicity. The lipophilic hexyl chain on CHED facilitates penetration into leather and the biocide associates with the fatty material in the leather. CHED exhibits a very low MIC (minimum inhibitory concentration) against the typical fungi found in the leather industry.[5]

**CHED Synthesis**

CHED is manufactured by combining cyanamide and carbon disulfide in the presence of potassium (sodium) hydroxide. The kabonate (nabonate)[6] is reacted with bromohexane in the presence of a phase transfer catalyst resulting in hexyl kabonate (nabonate). The final step in the reaction series involves reacting the aqueous solution of hexyl kabonate (nabonate) with bromochloromethane (BCM) to yield S-hexyl-S'-chloromethyl-cyanodithiocarbimate. Much work has been done recently on the synthesis parameters to minimize the production of by-products and maximise yields.

*Antifungal Assessment*

*a) Preparation of the test organism*

*Aspergillus niger* (ATCC 9642) wild-type was maintained on potato dextrose agar (PDA, Difco, Detroit, MI). Aliquots of a fungal spore suspension were used to challenge chemically-treated units of liquid fungal growth medium. Spore suspensions of *A. niger* were prepared by transferring spores from a viable culture on an agar slant to sterile deionized water using a sterile cotton swab. Spore suspensions were standardized by adjusting with sterile water such that its $OD_{686}$ = 0.28. This density provides approximately 2.5 x $10^7$ CFU $mL^{-1}$ of spore suspension.

*b) Determination of test compound antifungal activity*

The *in vitro* antifungal activity (MIC) was determined using a liquid growth medium added to sterile 18 x 150mm culture tubes. The mineral salts broth was prepared as described in ASTM G21-70[7], and amended with glucose (10g $L^{-1}$ and yeast extract (1g $L^{-1}$). The initial concentrations of stock solutions of each test compound were prepared such that a known µL aliquot delivered to 5mL of growth medium would provide a required test concentration as ppm product. Each treatment tube containing 5mL of growth medium was inoculated with 100µL of the standardized spore suspension of *A. niger*. Test units were incubated at 30°C for 4-7 days, and each culture tube was inspected for the presence/absence of fungal growth.

*c) Test method for interaction/synergy*

The test method used in this phase of the study was the basic *in vitro* fungicide screening protocol except using standard 96-well microplates rather than culture tubes. In all cases the experimental design was a factorial which is well-suited for use with the microplate. An aliquot (250µL) of sterile medium was dispensed into each test well of a standard 96-well microplate (Corning No. 430247). A series of multiple concentrations for compound A is introduced in one direction into microplate wells containing liquid growth medium. Similarly multiple concentrations for compound B are introduced in the second direction. In so doing each concentration of A is tested in combination with each concentration of B. Stock solutions of test compounds were prepared by dissolving the materials in 9:1 (v/v) solution of

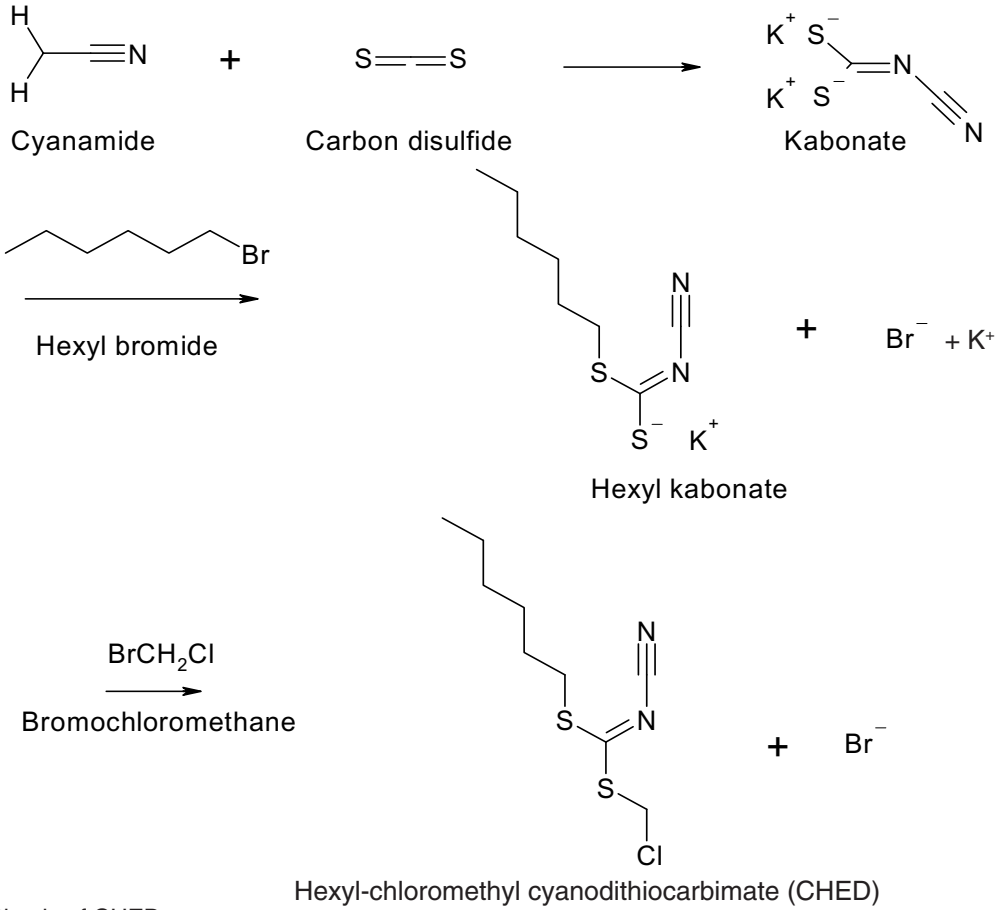


**Figure 1.** Synthesis of CHED.

acetone:methanol. Appropriate volumes of stock solutions were added to the test wells in order to achieve the desired ppm levels. Each test well (plus controls) was then inoculated with 5µL of a standardized suspension of *Aspergillus niger*. Each combination of CHED (A) with the second biocide (B) was conducted at least twice using duplicate factorial plates in each experiment. Plates were incubated at 30°C for 4-7 days. The optical density for each well at 686nm was then recorded automatically using a SpectraMax 340 microplate reader (Molecular Devices Corp., Sunnyvale, CA). All wells were visually inspected in order to corroborate data from the instrument. Test wells with an OD ≤0.05 were judged to exhibit complete inhibition of cellular growth.

*d) Synergy calculations:*

The nature of a potential interaction among test compounds was determined by applying the computational technique of Kull, *et al.*[8]

$$X = Q_A/Q_a + Q_B/Q_b$$

where:

$Q_a$ = lowest concentration (ppm) of compound A alone which inhibited growth

$Q_b$ = lowest concentration (ppm) of compound B alone which inhibited growth

$Q_A$ = lowest concentration (ppm) of compound A in the mixture which inhibited growth

$Q_B$ = lowest concentration (ppm) of compound B in the mixture which inhibited growth

For: X < 1 A and B are synergistic
X = 1 A and B are additive
X > 1 A and B are antagonistic

## RESULTS AND DISCUSSION

*a) MIC data for class analogues*

**TABLE I**
**Antifungal MIC (ppm ai) activities against *Aspergillus niger* for members of the class of S-Halo-(X) methyl-S'-aryl/alkyl-(R) cyanodithiocarbimates**

| X | R | Antifungal MIC |
|---|---|---|
| Cl | $-CH_2CHC=CH_2$ | 1.0 |
| | $-(CH_2)_7CH_3$ | 2.0 |
| | $-CH_3$ | 1.0 |
| | $-(CH_2)_{11}CH_3$ | 6.0 |
| (CHED) | $-(CH_2)_5CH_3$ | 0.2 |
| | $-(CH_2)_3CH_3$ | 0.4 |
| | $-(CH_2)_2CH_3$ | 0.5 |
| | $-CH_2C_6H_5$ | 1.0 |
| | $-CH_2(CH_3)_2$ | 0.5 |
| | $-(CH_2)_3OH$ | 6.0 |
| | $(CH_2CH_2O)_2$ | 6.0 |
| | $-(CH_2)_2OH$ | 6.0 |
| | $-(CH_2)_2CO_2H$ | >100 |
| Br | $-(CH_2)_3CH_3$ | 1.4 |
| | $-CH_2C_6H_5$ | 2.0 |
| I | $-(CH_2)_3CH_3$ | 2.0 |
| | $-CH_2C_6H_5$ | 5.0 |

*b) Synergy with a second biocide*

**TABLE II**
**Results of the application of the formula of Kull *et al.*, from MIC data generated by multiple factorial tests with CHED (A) and the listed second biocides (B)[9]**

| Biocide B | Test 1 | Test 2 | Test 3 |
|---|---|---|---|
| TCMTB | 0.27 | 0.25 | 0.7 |
| MTC[1] | 0.64 | 0.46 | 0.29 |
| BHAP[2] | 1.22 | 1.0 | 0.8 |
| BUSPERSE 2180[3] | 0.62 | 0.74 | --- |
| IPBC | 0.86 | 0.3 | --- |
| Propiconazole | --- | --- | 0.34 |
| DCOIT[4] | 0.7 | 0.5 | --- |
| OIT | 0.64 | --- | --- |
| BUSAN 1014[5] | 0.27 | 0.31 | 0.53 |
| PREVENTOL WB[5] | 0.84 | 0.22 | 0.21 |
| AMICAL[5] | 0.34 | 0.65 | 1.03 |
| BUSAN 1144[5] | 0.69 | 0.57 | --- |
| BUSAN 1210[5] | 0.59 | 0.61 | --- |

[1] Methylene (Bis) thiocyanate
[2] Bromohydroxyacetophenone
[3] Proprietary Potentiator
[4] 4, 5,-dichloro-2-N-octyl-4-isothiazolin-3-one
[5] Busan, Preventol, and Amical are proprietary fungicidal compositions of Buckman, LanXess and Dow Chemical companies respectively

**Industry performance data**

A significant amount of laboratory and field testing has taken place on CHED and synergistic combinations of CHED with other active substances to confirm performance under industry conditions. Standard Buckman evaluations for performance include analysis of treated leathers for actibve substance uptake and challenge testing in the Tropical Chamber (TC) for an extended period of 8 weeks. Testing to date has shown excellent efficacy with CHED on its own or in combination with other industry substances at reduced active substance and product offer

Table III shows average results from 6 separate laboratory tests run in India of a 30% CHED formulation against Busan 30L (30% TCMTB) as industry standard. At one third lower offer, the CHED formulation gave similar performance results on full thickness wet-blue with no mould growth after 8 weeks exposure in the Tropical Chamber.

**TABLE III**
**Results of laboratory trials on full thickness wet-blue - CHED (30% formulation) *versus* Busan 30L (TCMTB)**

| Product offer | Active substance[10] | TC[11]: 4 wks/8 wks |
|---|---|---|
| 0.1% Busan 30L | 61ppm | 10/10 |
| 0.07% CHED | 51ppm | 10/10 |

Table IV shows average results from 6 separate production drum trials run in Brazil where a synergistic combination of CHED (plus OIT and TCMTB) with total active substance concentration of 12.5% was run

against Busan 30L (30% TCMTB) as industry standard. The results indicate that at approximately 30% lower offer, the CHED combination product gave similar performance results on full thickness wet-blue after 8 weeks of exposure in the Tropical Chamber.

**TABLE IV:**
**Results of production scale trials on full thickness wet-blue - BLX-16085 (CHED, OIT, TCMTB) *versus* Busan 30L (TCMTB)**

| Product offer | Total active substance | TC: 4 wks/8 wks |
|---|---|---|
| 0.15% Busan 30L | 132ppm | 10/10 |
| 0.11% BLX-1608 | 73ppm | 10/10 |

## REGULATORY CONSIDERATIONS

Regulatory review has shown CHED to be as acceptable and in some cases it has proven more favourable than current industry active substances that are registered for use in leather manufacture in highly regulated regions like North America and Europe. These conclusions are based on standard toxicological testing and evaluation of the molecules environmental fate. Introduction of commercial products based on CHED will depend on local registration requirements as these vary considerably between countries. The process for full product registration in USA (FIFRA[12]) and Europe (BPD[13]) is the most comprehensive, very expensive, and takes many years to complete.

## CONCLUSIONS

Development of CHED and its synergistic formulations as fungicides for the long-term preservation of in-process wet leathers represents an important contribution to the global leather industry. For the last few decades there has not been an introduction of any new active substance as a viable alternate to the handful of compounds that work within the technical and commercial constraints of the industry.

Indications are that CHED, on its own or in combination with other industry actives, will provide an effective and reliable alternative for the tanner.


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**References**


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2. US Patent 7,157,017 (Fungicidal Compositions and methods using cyanodithiocarbimates) issued to Buckman Laboratories International, Inc. on January 2, 2007.
3. C. Fieseler, W. Walek and U. Thust., *Tag.-Ber. Akad. Landwirtsch.-Wiss.* der DDR, Berlin, 1990, **291**, 317-321.
4. German Patent DD 275433 C2 (cyanoimidodithiocarbonates as wood preservatives), W. Walek, J. Nauman, H. D. Pfeiffer, *et al.*
5. MIC = Minimum Inhibitory Concentration is the lowest concentration of the test chemical that completely inhibits the appearance of fungal growth on the surface of the growth medium.
6. Kabonate is the Buckman trade name for the dipotassium salt of cyanodithiocarbimate. Nabonate is the trade name for the disodium salt of cyanodithiocarbimate.
7. ASTM G21-70 (Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi). In *ASTM Standards on Materials and Environmental Microbiology*, Storer, R. A., Ed. American Society for Testing and Materials, Philadelphia, 1st ed, pp 180-3. (1987).
8. Kull, F. C., Eisman, P. C., Sylwestrowicz, H. D. *et al.,* Mixtures of quaternary ammonium compounds and long-chain fatty acids as antifungal agents. *Applied Microbiology*, 1961, **9**, 538-541.
9. Results are the subject of US Patent Application # 11/555,301.
10. Standard Buckman method: Solvent extraction of active substance followed by HPLC separation and quantification using UV detection against known standards. Values are corrected to 5mm wet-blue thickness and 60% average moisture content.
11. Standard Buckman method: Tropical chamber challenge test using optimum growth conditions and inoculation with known and wild strains of fungi. Leather sample coupons hung in the chamber are read weekly. A value of 10 indicates no fungal growth.
12. Federal Insecticide, Fungicide and Rodenticide Act of 1947 and its amendments.
13. Biocidal Products Directive 98/8/EC and its amendments.