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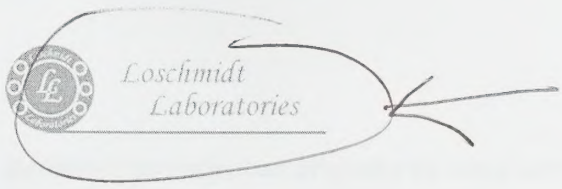

METHOD OF DETOXIFICATION OF YPERITE BY USING HALOALKANE DEHALOGENASES

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Abstract: The method of detoxification of yperite by the use of haloalkane dehalogenases or their compositions, the method of preparation of dehalogenating enzymes and of decontamination compositions which contain at least one wild type and/or modified haloalkane dehalogenase (EC 3.8.1.5) as an chemically active component. The preferred halogenases are DhaA from *Rhodococcus rhodochrous* NCIMB 13064, DmbA from *Mycobacterium bovis* 5033/66 or LinB from *Sphingomonas paucimobilis* UT26. Decontamination is utilized for detoxification of yperite from the surfaces of instrumentality, constructional objects, the people's or animal's skin and elements of environment, when yperite is exposed to the action of decontamination composition at +10 °C to +70 °C, preferably at +40 °C and pH = 4 to 12.

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Method of detoxification of yperite by using haloalkane dehalogenases

Field of the invention

This invention relates to method for detoxification of yperite by using haloalkane dehalogenases (Enzyme Commission number EC 3.8.1.5) as a primary, chemically active component of decontamination compositions. Decontamination compositions are designated for detoxification of yperite (2,2'-dichlorodiethylsulfide) on the surface of military hardware, transportational, industrial and agricultural hardware, technical devices and constructional objects (hereafter instrumentation), people's or animal's skin and elements of environment (water, soil, sediments and air), that are contaminated by this highly toxic blistering substance.

State of the art

At the present time, there are being used decontamination compositions in the armed forces, civil defense troops, fire services and rescue forces, that exhibit high unit consumption and undesirable aggressiveness on material, because their chemically active components are stechiometric agents, that are gradually consumed during their reaction with yperite. Their application on instrumentation leads to depreciation of decontaminated material or surfaces by corrosion and if these compositions get into soil or water, it endangers environment.

There have been described enzymes in the literature that exhibit activity against highly toxic organophosphorous (neural) substances, called organophosphorous hydrolases, OPA anhydrases or DFPases. As the only example of biological detoxification of blistering yperite (2,2'-dichlorodiethylsulfide) the use of bacteria species *Rhodococcus rhodochrous* IGTS8 (ATCC 53968) was mentioned in the art so far, which has the ability to utilize a chemical analog of yperite 2-chlorethyl-ethylsufite as the only source of carbon for it's growth [Kilbane, J. J., and Jackowski, K. (1996) *J. Chem. Tech. Biotechnol.* 65, 370-374]. Detoxification activity of bacteria species *Rhodococcus rhodochrous* IGTS8 (ATCC 53968) is based on splitting the S-C bond in the molecule. The application of the enzyme splitting C-S bond in a non-toxic product of hydrolysis, thiodiglykol, has been published [Harvey, S., DeFrank, J. J., Valdes, J. J., Kamely, D, and Chakrabarty, A. M., (1990) *Proceedings: Biotechnology-Biodegradation Workshop Symposium by US Army Research Office,* 47-58; Kilbane, J. J., (1990) *Resources Conserv. and Recycl.* 3, 69-79].

Haloalkane dehalogenases are enzymes able to remove halogen from halogenated aliphatic compound by a hydrolytic replacement, forming the corresponding alcohols [Janssen, D. B. Pries, F., and Van der Ploeg, J. R. (1994) *Annual Review of Microbiology* 48, 163-191]. Hydrolytic dehalogenation proceeds by formal nucleophilic substitution of the halogen atom by

hydroxyl ion. Structurally, haloalkane dehalogenases belong to the α/β-hydrolase fold superfamily [Ollis, D. L., Cheah, E., Cygler, M., Dijkstra, B., Frolow, F., Franken, S. M., Harel, M., Remington, S. J., Silman, I., Schrag, J., Sussman, J. L., Verschueren, K. H. G., and Goldman, A. (1992) *Protein Engineering* 5, 197-211]. Haloalkane dehalogenases contain a nucleophile elbow [Damborsky, J. (1998) *Pure and Applied Chemistry* 70, 1375-1383] that belongs to the most conserved structural features of the α/β-hydrolase fold. Another highly conserved region in haloalkane dehalogenases is the central β-sheet that is flanked on both sides by α-helixes that form hydrophobic core of the main domain. The main domain carries the catalytic triad Asp-His-Asp/Glu. The second domain is solely consisting of α-helixes and is placed like a cap on top of the main domain. Interface between the main and the cap domain forms the active site of the enzyme. Whereas there is significant similarity between main domains, the sequence and structure of the cap domain varies significantly in different haloalkane dehalogenases. It is supposed, that character and structure of the cap domain radically determine the substrate specificity [Pries, F., Van den Wijngaard, A. J., Bos, R., Pentenga, M., and Janssen, D. B. (1994) *Journal of Biological Chemistry* 269, 17490-17494; Kmunicek, J., Luengo, S., Gago, F., Ortiz, A. R., Wade, R. C., and Damborsky, J. (2001) *Biochemistry* 40, 8905-8917].

Objects and summary of the invention

By the method of this invention, the above mentioned imperfections of existing decontamination compositions, that consist of stechiometric agents, are to the great extent overcame by preparations, that consist of catalyst of hydrolytic detoxification of yperite, that is enzyme or mixture of dehaloalkane dehalogenases. The main and significant component of the compositions is the presence of at least one enzyme of the haloalkane dehalogenases. In general this method includes hydrolytic dehalogenation of yperite in the way, that decontamination composition consisting of one or more wild or modified haloalkane dehalogenases affect yperite and convert it to non-toxic product thiodiglycol. Haloalkane dehalogenase is expressed in the natural producer or in a heterologous host organism, e.g. in bacteria *Escherichia coli,* or in yeast *Pichia pastoris.* The enzyme used, can be in non-living or living cells, in the form of crude extract or purified protein. As an enzyme for the dehalogenase composition at least one haloalkane dehalogenase selected from the family of enzymes EC 3.8.1.5 is used, e.g. haloalkane dehalogenase DhaA from *Rhodococcus rhodochrous* NCIMB 13064, DmbA from *Mycobacterium bovis* 5033/66 or LinB from *Sphingomonas paucimobilis* UT26.

Haloalkane dehalogenases constitute an important group of enzymes that are able to cleave the halogen-carbon bond in halogenated aliphatic compounds. They exhibit a broad substrate specificity including haloalkanes, haloalkenes, haloethers and haloalcohols. The mechanism of dehalogenation is based on the nucleophilic attack of the carbon atom to which the halogen is bound and proceeds to cleavage of halogen ion and formation of alkyl-enzyme intermediate. The intermediate is subsequently hydrolyzed with production of corresponding alcohol, halogen ion and proton. The enzyme haloalkane dehalogenase transforms yperite into non-toxic bis(2-hydroxyethyl)sulfide by hydrolytic dehalogenation.

In the decontamination compositions haloalkane dehalogenase can be in crude or purified extract, immobilized on a carrier material, free in aqueous solution, in a monophasic organic or aqueous solution or in organic/aqueous biphasic systems. Enzymes can be immobilized by absorption on the inorganic or organic carrier material (such as: Celite, activated charcoal, aluminium oxide, cellulose, synthetic resins, Sephadex) or covalent attachment onto the surface of organic material (such as: cellulose, dextran, starch, chitin, agarose) inorganic material (such as: porous glass), or synthetic polymeric carrier material (such as: VA-Epoxy Biosynt, Eupergit).

The enzyme haloalkane dehalogenase may be dissolved, crystalline, lyophilized or precipitated. The enzyme can be confined to a restricted area, where it remains catalytically active – entrapped into a solid matrix or into by a membrane restricted compartments. Enzymes may be entrapped into a biological matrix, e.g., agar gel, alginate gel, κ-carragenan. The enzyme can be entrapped also to inorganic stable matrices, e.g., silica gel. A tight network that is able to carry isolated enzyme can be obtained by polymerization of synthetic monomers, e.g., polyacrylamide, in the presence of the enzyme. Depending on the immobilization technique, the properties of the enzyme such as catalytic rate, stability and binding affinity may be significantly altered. The hydrolytic detoxification of yperite catalysed by the enzyme can be performed at the temperature range 10 – 70 °C with reaction optimum around 40 °C.

Additional components are aqueous buffer systems (e.g., phosphate buffer, Tris-sulfate buffer, glycine buffer, acetate buffer or citrate buffer) which stabilize the neutral pH being close to optimum interval of 7.0 – 8.5. The pH activity profile is broader and allows pH interval from 4 to 12 while maintaining a reasonable activity. Another additional components are surfactants or organic solvents, that facilitate dissolving /dissolution of yperite in aqueous solvents. Addition of water-miscible organic solvents, e.g., methanol, tert-butanol, aceton, dioxane, acetonitrile, dimethyl formamide, dimethyl sulfoxide, tetrahydrofuran, 3-methyl-3-pentanol and pyridine, can be used at concentration up to 70 % of the total volume depending on the enzyme stability.

Decontamination compositions based on haloalkane dehalogenases can consist of two macroscopic phases, namely the aqueous phase containing the dissolved enzyme and a second phase of organic solvents, partially water soluble or insoluble in water, e.g., ethyl acetate, diethyl ether, methyl tert-butyl ether, cyclohexanol, n-propylacetate, ethyl chloroacetate, bis(2-chloroethyl)ether, isopropyl acetate, butyl acetate, isobutyl acetate, hexanol, isoamyl acetate, n-amyl acetate, toluene, octanol, isoheptane, n-butyl ether, cyclohexane, 2-methylpentane, n-hexane, methylcyclohexane a n-octane. Organic phase enhances solubility of yperite in the decontamination composition which penetrates into water phase. The reaction takes place in aqueous phase, where the enzyme is in natural environment and is not in direct contact with organic solvent, where the most of dissolved yperite is located. The transfer of reactant and product between the two phases, reactant to the enzyme, product from the enzyme, can be increased by enlarging the surface between the two phases (producing a fine dispersion) or by stirring. The bulk water can be replaced by addition of water immiscible organic solvent. The enzyme is than suspended in a monophasic organic solvent. The optimum catalytic activity of the enzyme in organic solvent can be obtained by adjustment and maintenance the water content. This can be conventionally obtained by a pair of salt/hydrate, e.g., $CaCl_2 \cdot H_2O/2\ H_2O$, NaI anh./2 H_2O, Na_2HPO_4 anh./2 H_2O, NaOAc anh./3 H_2O, NaBr anh./2 H_2O, $Na_4P_2O_7$ anh./7 H_2O, $Na_2HPO_4 \cdot 2\ H_2O/7\ H_2O$, Na_2SO_4 anh./10 H_2O, $Na_2HPO_4 \cdot 7\ H_2O/12\ H_2O$, that are added to the solvent and function as a water buffer. The enzyme solubility in lipophilic organic solvents can be modified by covalent attachment of the amphiphatic polymer polyethylene glycol to the surface of enzyme. Linkage of the polymer chain onto the enzyme surface is achieved by reaction of ε-amino groups of lysine residues with „linker", e.g., cyanuric chloride. Protein stabilizers such as polyalcohols, e.g., sugar alcohols or glycerol, inactivated proteins, e.g., bovine serum albumin, or polymers, which show a certain structural resemblance with water, e.g., polyethylene glycol, polyvinyl alcohol, can be added to the reaction medium to enhance the enzyme stability.

Haloalkane dehalogenases used according to this invention can be additionally produced by means of rational design based on structural analysis, e.g., protein crystallography, nuclear magnetic resonance and circular dichroism spectroscopy, and biochemical characterization, e.g., steady-state kinetics, transient kinetics, stability and thermo stability assays, spectroscopic analyses and a like, followed by computer modelling, e.g., sequence comparisons, phylogenetic analysis, homology modelling, molecular docking, molecular mechanics, molecular dynamics, quantum mechanics and multivariate statistics, and DNA mutagenesis, e.g., cassette mutagenesis, site-directed mutagenesis, chemical mutagenesis, error-prone PCR, site saturation mutagenesis, ensemble mutagenesis, recursive ensemble mutagenesis, scanning saturation mutagenesis, mutator strains, etc. The procedure

includes altering at least one amino acid residue of the haloalkane dehalogenase with another amino acid residue or recombining two or more members of the haloalkane dehalogenases to obtain a modified enzyme with improved efficacy. Modified nucleic acids can be introduced into a cell, in which they can be expressed to provide an altered haloalkane dehalogenase.

Advantages of decontamination compositions with haloalkane dehalogenases are, that

a) It posses desired detoxification activity on the yperite, which is homogenously dissolved in aqueous solutions with pH in the range from 4 to 12 (with the neutral optimum of pH 7.0 to 8.5) at the temperature range +0 to +70°C with reaction optimum around +40 °C.

b) A low initial concentration (1×10^{-5} mol.l^{-1}) is suitable to attain desired reaction rate, that means a satisfactory level of detoxification in about 15 to 30 minutes.

c) It exhibits catalytic activity, it stays unexhausted during reaction, which brings savings/cost reduction in logistic area.

d) It doesn't exhibit chemical aggressiveness against common construction materials and components of technique that resist the affect of neutral aqueous, corrosively non-aggressive decontamination compositions (aqueous suspensions, foams, emulsions or micro-emulsions).

e) It doesn't exhibit any toxicity and is degradable in environment

Examples of implementation

The enzyme is prepared by homologous expression in native organism/natural producer or by heterologous expression in a host organism, e.g., bacterium *Escherichia coli* or yeast *Pichia pastoris*. According to the invention, the enzyme present in living or non-living cells is used in the form of crude extract or purified protein.

Example 1

To overproduce wild type of enzyme haloalkane dehalogenase LinB from *Sphingomonas paucimobilis* UT26 (Sequence 1) the corresponding gene was cloned in the pPICZαA expressional vector. Cloned plasmids were than transferred into *Pichia pastoris* GS115. *Pichia pastoris* GS115 was than cultured at 28°C in growth medium (1 weight % of yeast extract, 2 wgt % of peptone, 4×10^{-5} wgt % of Biotine and 1 wgt % of casamino acid in 100 mM potassium phosphate buffer, pH 6.5). The induction of the enzyme synthesis was

initiated by addition of 0.7 volume % of methanol when the culture reached an optical density of 2 at 600 nm. After induction the culture was incubated at 28 °C for 10 h and then harvested. Ammonium sulfate was added to supernatant to a final concentration of 75% of saturation. Solution was stirred 30 min until the added ammonium sulfate was dissolved. The supernatant was centrifugated 15 min at 11 000 *g*. Pellet was than re-suspended in 20 mM potassium phosphate buffer, pH 7.5 with content of 0.5 M sodium chloride and 10 mM imidazole. The haloalkane dehalogenase was then purified on a Ni-NTA Sepharose column HR 16/10 (Qiagen, Germany). The His-tagged haloalkane dehalogenase was bound to the resin in the equilibrating buffer, which contained 20 mM potassium phosphate buffer pH 7.5; 0.5 M sodium chloride and 10 mM imidazole. Unbound and weekly bound proteins were washed off by buffer containing 60 mM imidazole. Then the His-tagged enzyme LinB was eluted by buffer containing 160 mM imidazole. The active fractions were dialyzed overnight against 50 mM potassium phosphate buffer, pH = 7.5. The enzyme was stored at 4 °C in 50 mM potassium phosphate buffer, pH 7.5, containing 10 % glycerol and 1 mM 2-mercaptoethanol to enhance long-lasting enzyme stability.

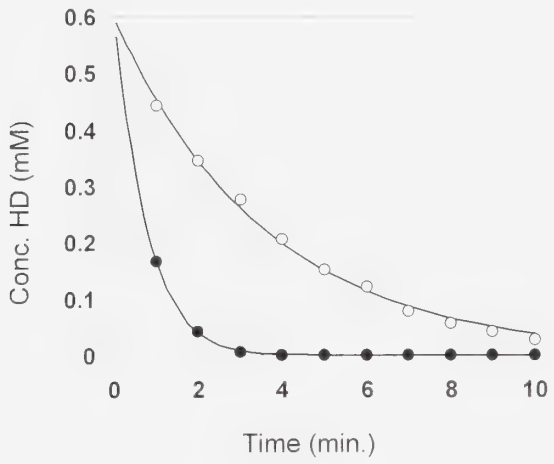


Fig. 1 – Conversion of yperite by the use of haloalkane dehalogenase LinB. Spontaneous hydrolysis of yperite without enzyme k_c = 0.0046 s^{-1} (empty circles) and degradation of yperite in the presence of haloalkane dehalogenaze LinB k_{cat}/K_m = 6.9 $s^{-1}.mM^{-1}$ (black/filled circles).

Hydrolytic dehalogenation catalyzed by haloalkane dehalogenase (wild type or modified) converts the toxic yperite into non-toxic bis(2-hydroxyethyl)sulfite. The hydrolytic dehalogenation of yperite was catalyzed by haloalkane dehalogenase at 37 °C in 50 mM phosphate buffer (pH 7.5; adjusted by addition of 1M NaOH solution). The yperite was added into reaction buffer to its final concentration in buffer of 94.3 $mg.l^{-1}$ of yperite. Reaction was initiated by addition of solution of enzyme LinB (50 mM potassium phosphate buffer pH 7.5; 1×10^{-5} to 1×10^{-4} $mol.l^{-1}$ of haloalkane dehalogenase LinB, 10 vol % of glycerol and 1 $mmol.l^{-1}$ 2-mercaptoethanol). Operation of haloalkane dehalogenase leads to rapid and complete decontamination of the yperite. The kinetics of reaction is shown in Figure 1. Catalytic activity/power of haloalkane dehalogenase LinB by decontamination of yperite is $k_{cat}/K_m = 6.9\ s^{-1}.mM^{-1}$.

Sequence 1. Sequence of the gene *linB* and haloalkane dehalogenase LinB isolated from bacterium *Sphingomonas paucimobilis* UT26.

atg agc ctc ggc gca aag cca ttt ggc gag aag aaa ttc att gag atc aag ggc cgg cgc atg gcc tat atc gat gaa ggg acc ggc gat ccg atc ctc ttc cag cac ggc aat ccg acg tcg tcc tat ctg tgg cgc aat atc atg ccg cat tgc gcc ggg ctg gga cgg ctg atc gcc tgt gac ctg atc ggc atg ggc gat tcg gac aag ctc gat ccg tcg ggg ccc gag cgt tat gcc tat gcc gag cat cgt gac tat ctc gac gcg ctg tgg gag gcg ctc gat ctc ggg gac agg gtt gtt ctg gtc gtg cat gac tgg ggg tcc gcc ctc ggc ttc gac tgg gcc cgc cgc cac cgc gag cgt gta cag ggg att gcc tat atg gaa gcg atc gcc atg ccg atc gaa tgg gcg gat ttt ccc gaa cag gat cgc gat ctg ttt cag gcc ttt cgc tcg cag gcg ggc gaa gaa ttg gtg ttg cag gac aat gtt ttt gtc gaa caa gtt ctc ccc gga ttg atc ctg cgc ccc tta agc gaa gcg gag atg gcc gcc tat cgc gag ccc ttc ctc gcc gcc ggc gaa gcc cgt cga ccg acc ctg tct tgg cct cgc caa atc ccg atc gca ggc acc ccg gcc gac gtg gtc gcg atc gcc cgg gac tat gcc ggc tgg ctc agc gaa agc ccg att ccg aaa ctc ttc atc aac gcc gag ccg gga gcc ctg acc acg ggc cga atg cgc gac ttc tgc cgc aca tgg cca aac cag acc gaa atc acg gtc gca ggc gcc cat ttc atc cag gag gac agt ccg gac gag att ggc gcg gcg att gcg gcg ttt gtc cgg cga ttg cgc cca gca taa

MSLGAKPFGEKKFIEIKGRRMAYIDEGTGDPILFQHGNPTSSYLWRNIMPHCAGLGRLIACDLI
GMGDSDKLDPSGPERYAYAEHRDYLDALWEALDLGDRVVLVVHDWGSALGFDWARRHRER
VQGIAYMEAIAMPIEWADFPEQDRDLFQAFRSQAGEELVLQDNVFVEQVLPGLILRPLSEAEM
AAYREPFLAAGEARRPTLSWPRQIPIAGTPADVVAIARDYAGWLSESPIPKLFINAEPGALTTG
RMRDFCRTWPNQTEITVAGAHFIQEDSPDEIGAAIAAFVRRLRPA

Example 2

To overproduce enzyme haloalkane dehalogenase DhaA from *Rhodococcus rhodochrous* NCIMB 13064 (Sequence 2), the corresponding gene was cloned in the pYBJA2 vector containing *tac* promotor (P_{tac}) under the control of *lacI*^{q}. *Escherichia coli* BL21 containing pAQN plasmid was cultured in 250 ml Luria broth at 37 °C. The induction of enzyme synthesis was initiated by addition of isopropyl-β-D-thiogalactopyranoside to a final concentration of 0.5 mM when the culture reached an optical density of 0.6 at 600 nm. After induction, the culture was incubated at 30°C for 4 h and then harvested. The cells were disrupted by sonication using a Soniprep 150 (Sanyo, UK). The supernatant was used after centrifugation at 20000 *g* for 1 h. The haloalkane dehalogenase was purified on a Ni-NTA Sepharose column HR 16/10 (Qiagen, Germany). His-tagged haloalkane dehalogenase was bound onto the resin in the equilibrating buffer, which contained 20 mM potassium phosphate buffer pH 7.5, 0.5 M sodium chloride and 10 mM imidazole. Unbound and weakly bound proteins were washed off by buffer containing 60 mM imidazole. The His-tagged haloalkane dehalogenase was then eluted by buffer containing 160 mM imidazole. The active fractions were dialysed overnight against 50 mM potassium phosphate buffer, pH 7.5. The enzyme was stored at 4 °C in 50 mM potassium phosphate buffer, pH 7.5, containing 10 % glycerol and 1mM 2-mercaptoethanol enhancing long-lasting enzyme stability.

Enzyme haloalkane dehalogenase DhaA or its variant is the part of decontamination composition that contains aforementioned enzyme at concentration 1×10^{-6} to 1×10^{-4} mol.l^{-1}, further 1 to 5 vol % of aliphatic hydrocarbon of general formula C_nH_{2n+2} or cyclic aliphatic hydrocarbon of general formula C_nH_{2n}, where n is 6 to 12, further 5 to 20 vol % of aliphatic alcohol of general formula $C_nH_{2n+1}OH$, where n is 2 to 4, further 3 to 15 wgt % of anion active tenside of general formula $C_nH_{2n+1}OSO_3Me$, where n is 10 to 16 and Me stands for counter ion (Na^+, K^+ or monoethanol amonium), 1 to 10 wgt % alkylbenzensulfonate of general formula $R^{(3)}$-$(Ar)SO_3^-.Me^+$, where $R^{(3)}$ stands for alkyl with 11 to 13 atoms of carbon, and Me^+ indicates sodium ion, further components of glycine buffer to adjust pH of aqueous solution in the range from 7 to 9, or else glycine of total concentration 0.1 mol.l^{-1}, etc. Required pH 8.2 is reached by addition of 1M NaOH. The rest to make 100% is water. The catalytic power of haloalkane dehalogenase DhaA at decontamination of yperite is k_{cat}/K_m = 5.7 s^{-1}.mM^{-1}.

Sequence 2. Sequence of the gene *dhaA* and haloalkane dehalogenase DhaA isolated from bacterium *Rhodococcus rhodochrous* NCIMB 13064.

atg tca gaa atc ggt aca ggc ttc ccc ttc gac ccc cat tat gtg gaa gtc ctg ggc gag cgt atg cac tac gtc gat gtt gga ccg cgg gat ggc acg cct gtg ctg ttc ctg cac ggt aac ccg acc tcg tcc tac ctg tgg cgc aac atc atc ccg cat gta gca ccg agt cat cgg tgc att gct cca gac ctg atc ggg atg gga aaa tcg gac aaa cca gac ctc gat tat ttc ttc gac gac cac gtc cgc tac ctc gat gcc ttc atc gaa gcc ttg ggt ttg gaa gag gtc gtc ctg gtc atc cac gac tgg ggc tca gct ctc gga ttc cac tgg gcc aag cgc aat ccg gaa cgg gtc aaa ggt att gca tgt atg gaa ttc atc cgg cct atc ccg acg tgg gac gaa tgg ccg gaa ttc gcc cgt gag acc ttc cag gcc ttc cgg acc gcc gac gtc ggc cga gag ttg atc atc gat cag aac gct ttc atc gag ggt gcg ctc ccg aaa tgc gtc gtc cgt ccg ctt acg gag gtc gag atg gac cac tat cgc gag ccc ttc ctc aag cct gtt gac cga gag cca ctg tgg cga ttc ccc aac gag ctg ccc atc gcc ggt gag ccc gcg aac atc gtc gcg ctc gtc gag gca tac atg aac tgg ctg cac cag tca cct gtc ccg aag ttg ttg ttc tgg ggc aca ccc ggc gta ctg atc ccc ccg gcc gaa gcc gcg aga ctt gcc gaa agc ctc ccc aac tgc aag aca gtg gac atc ggc ccg gga ttg cac tac ctc cag gaa gac aac ccg gac ctt atc ggc agt gag atc gcg cgc tgg ctc ccc gca ctc tag

MSEIGTGFPFDPHYVEVLGERMHYVDVGPRDGTPVLFLHGNPTSSYLWRNIIPHVAPSHRCIA
PDLIGMGKSDKPDLDYFFDDHVRYLDAFIEALGLEEVVLVIHDWGSALGFHWAKRNPERVKGI
ACMEFIRPIPTWDEWPEFARETFQAFRTADVGRELIIDQNAFIEGALPKCVVRPLTEVEMDHYR
EPFLKPVDREPLWRFPNELPIAGEPANIVALVEAYMNWLHQSPVPKLLFWGTPGVLIPPAEAA
RLAESLPNCKTVDIGPGLHYLQEDNPDLIGSEIARWLPAL

Example 3

To overproduce enzyme haloalkane dehalogenase DmbA from *Mycobacterium bovis* 5033/66 (Sequence 3), the same procedure as in example 1 is followed. Enzyme haloalkane dehalogenase DmbA or its variant is the part of decontamination composition that contains aforementioned enzyme at concentrations 1×10^{-6} to 1×10^{-4} mol.l^{-1}, further 1 to 20 vol % of aliphatic alcohol of general formula $C_nH_{2n+1}OH$, where n is 2 to 4, further 3 to 15 wgt % of anion active surfactant of general formula $C_nH_{2n+1}OSO_3Me$, where n is 10 to 16 and Me stands for counter ion (Na^+, K^+ or monoethanol amonium), 1 to 10 wgt % of ethoxy nonylphenol of general formula C_9H_{19}-Ar-O-$(C_2H_4O)_nH$, where n is 9 to 10, further components of glycine buffer to adjust pH of aqueous solution in the range from 7 to 9, or else glycine of total concentration 0.1 mol.l^{-1}, etc. Required pH is reached by addition of 1M NaOH. The rest to

make 100% is water. The catalytic power of haloalkane dehalogenase DmbA at decontamination of yperite is k_{cat}/K_m = 6.0 $s^{-1}.mM^{-1}$.

Sequence 3. Sequence of the gene *dmbA* and haloalkane dehalogenase DmbA isolated from bacterium *Mycobacterium bovis* 5033/66.

atg aca gca ttc ggc gtc gag ccc tac ggg cag ccg aag tac cta gaa atc gcc ggg aag cgc atg gcg tat atc gac gaa ggc aag ggt gac gcc atc gtc ttt cag cac ggc aac ccc acg tcg tct tac ttg tgg cgc aac atc atg ccg cac ttg gaa ggg ctg ggc cgg ctg gtg gcc tgc gat ctg atc ggg atg ggc gcg tcg gac aag ctc agc cca tcg gga ccc gac cgc tat agc tat ggc gag caa cga gac ttt ttg ttc gcg ctc tgg gat gcg ctc gac ctc ggc gac cac gtg gta ctg gtg ctg cac gac tgg ggc tcg gcg ctc ggc ttc gac tgg gct aac cag cat cgc gac cga gtg cag ggg atc gcg ttc atg gaa gcg atc gtc acc ccg atg acg tgg gcg gac tgg ccg ccg gcc gtg cgg ggt gtg ttc cag ggt ttc cga tcg cct caa ggc gag cca atg gcg ttg gag cac aac atc ttt gtc gaa cgg gtg ctg ccc ggg gcg atc ctg cga cag ctc agc gac gag gaa atg aac cac tat cgg cgg cca ttc gtg aac ggc ggc gag gac cgt cgc ccc acg ttg tcg tgg cca cga aac ctt cca atc gac ggt gag ccc gcc gag gtc gtc gcg ttg gtc aac gag tac cgg agc tgg ctc gag gaa acc gac atg ccg aaa ctg ttc atc aac gcc gag ccc ggc gcg atc atc acc ggc cgc atc cgt gac tat gtc agg agc tgg ccc aac cag acc gaa atc aca gtg ccc ggc gtg cat ttc gtt cag gag gac agc cca gag gaa atc ggt gcg gcc ata gca cag ttc gtc cgg cag ctc cgg tcg gcg gcc ggc gtc tga

MTAFGVEPYGQPKYLEIAGKRMAYIDEGKGDAIVFQHGNPTSSYLWRNIMPHLEGLGRLVAC
DLIGMGASDKLSPSGPDRYSYGEQRDFLFALWDALDLGDHVVLVLHDWGSALGFDWANQHR
DRVQGIAFMEAIVTPMTWADWPPAVRGVFQGFRSPQGEPMALEHNIFVERVLPGAILRQLSD
EEMNHYRRPFVNGGEDRRPTLSWPRNLPIDGEPAEVVALVNEYRSWLEETDMPKLFINAEPG
AIITGRIRDYVRSWPNQTEITVPGVHFVQEDSPEEIGAAIAQFVRQLRSAAGV

Example 4

Enzyme haloalkane dehalogenase LinB or its variant is the part of decontamination composition that contains the enzyme at concentrations 1×10^{-6} to 1×10^{-4} $mol.l^{-1}$, further 1 to 15 wgt % of anion active surfactant of general formula $C_nH_{2n+1}OSO_3Me$, where n is 10 to 16 and Me stands for protiion (Na^+, K^+ or monoethanol amonium), 1 to 10 wgt % of ethoxylated nonylphenol of general formula C_9H_{19}-Ar-O-$(C_2H_4O)_nH$, where n is 9 to 10, further components of phosphate buffer to adjust pH of aqueous solution in the range from 7 to 8.5; that is KH_2PO_4 a K_2HPO_4 in the required ratio and in a total concentration 50 $mmol.l^{-1}$, etc. The rest to make 100% is water.

Industrial utility

This invention is utilized in industry to eliminate yperite from the surfaces of military hardware, transportational, industrial and agricultural hardware, technical devices and constructional objects, of the people's or animal's skin and elements of environment, that are contaminated by this highly toxic blistering substance. This technology is utilized in armed forces and also in civil services, generally there, where is need to use decontamination compositions to decontaminate blistering substances.

CLAIMS

1. The method of detoxification of yperite by the use of haloalkane dehalogenases, determined by that, that yperite is exposed to at least one haloalkane dehalogenase selected from the group of enzymes with EC 3.8.1.5, with concentration of enzyme 1×10^{-6} 1×10^{0} mol.l^{-1}, at temperature from +10 °C to +70 °C and pH = 4 to 12 in liquid medium.

2. The method according to claim 1, determined by that, that haloalkane dehalogenase is an enzyme selected from the group containing dehalogenase DhaA from *Rhodococcus rodochrous* NCIMB 13064, DmbA from *Mycobacterium bovis* 5033/66, LinB from *Sphingomonas paucimobilis* UT26.

3. The method according to claim 1 and 2, determined by that, that used haloalkane dehalogenase is wild type or modified haloalkane dehalogenase or their mixture.

4. The method according to claims 1 to 3, determined by that, that the preparation of haloalkane dehalogenase is carried out in the presence of at least one protein stabilizer, selected from the group containing polyalcohols, inactive proteins, or polymers.

5 The method according to claims 1 to 4, determined by that, that the liquid medium is an organic solvent, a mono-phasic aqueous solution of an organic solvent, a biphasic system of organic and aqueous components.

6. The method according to claims 1 to 5, determined by that, that enzyme haloalkane dehalogenase is used in soluble form or crystalline or lyophilized or precipitated form.

7. The method of detoxification of yperite according to claims 1 to 6, determined by that, that enzyme haloalkane dehalogenase is immobilized by absorption or by ionic binding or by covalent attachment onto the surface of a carrier.

8. The method of detoxification of yperite according to claims 1 to 6, determined by that, that enzyme haloalkane dehalogenase is immobilized by cross-linking (linkage to each other) or entrapping enzyme into a solid matrix or a compartment confined by a membrane.

9. The method according to claims 1 to 8, determined by that, that detoxification is carried out in the presence of surfactants.

Abstract

Title: Method of detoxification of yperite by using haloalkane dehalogenases

The method of detoxification of yperite by the use of haloalkane dehalogenases or their compositions, the method of preparation of dehalogenationating enzymes and of decontamination compositions which contain at least one wild type and/or modified haloalkane dehalogenase (EC 3.8.1.5) as an chemically active component. The preferred dehalogenases are DhaA from *Rhodococcus rhodochrous* NCIMB 13064, DmbA from *Mycobacterium bovis* 5033/66 or LinB from *Sphingomonas paucimobilis* UT26. Decontamination is utilized for detoxification of yperite from the surfaces of instrumentality, constructional objects, the people's or animal's skin and elements of environment, when yperite is exposed to the action of decontamination composition at +10 °C to +70 °C, preferably at +40 °C and pH = 4 to 12.

DR. ALFRED BADER CBE
2A Holmesdale Road
Bexhill-on-Sea
East Sussex TN39 3QE
England
Phone/Fax: 44-1424-222223

A Chemist Helping Chemists

Ms. Sue Threlfall
Lancaster Synthesis 01524 39727

Dear Ms Threlfall:

You are easier to reach than Dr. Shaw!

I'll be in London the week of December 11, and will return to Milwaukee on December 22

Perhaps Dr. Shaw could meet me in Milwaukee when next he visits your people here.

Best regards

Alfred Bader

18 XI 00

Registered in England
No. 1080172

Registered Office: Lancaster Synthesis Ltd
Hayes Road, Cadishead, Manchester M44 5BX, UK

FAX

Subject: Re: Suggested Speakers for Symposium
From: Clint Lane <clint.lane@NAU.EDU>
Date: Mon, 07 Jun 2004 19:34:45 -0700
To: Alfred Bader Fine Arts <baderfa@execpc.com>
CC: "P. V. Ramachandran" <chandran@purdue.edu>

Dear Alfred,

Thank you for your thoughtful reply. I have also received a reply from Chandran and based on his response and your suggestions, the following is my revised list of potential speakers in alphabetical order:

Ronald Breslow
E. J. Corey
Dennis Curran
Samuel Danishefsky
S. G. Davies
K. C. Nicolaou
K. Barry Sharpless
Victor A. Snieckus

I will soon send an invitation to each of the above by e-mail. You will note that I have deleted Evans and Wender (deletion suggested by Chandran) and have added Snieckus and Nicolaou. We probably will need to limit the number of speakers to a total of 8. It is expected that some these individuals may not be available to speak. Thus, I am confident Chandran would welcome the opportunity to speak and can always be added if needed. We might even be able to expand the list to 9 and add Chandran even if all 8 accept our invitation. I do agree it would be nice to invite our old friend Jim Cook, and I will put him at the top of my back-up list in case one or more of our top 8 decline the invitation.

Thank you again for your suggestions, and I will keep you informed of our progress as we move forward with the details for this symposium.

Best regards,
Clint

At 11:49 AM 06/07/2004 -0500, you wrote:

> Please consider adding one of our good friends from Purdue, perhaps Professor Ramachandran or Jim Cook at UWM or Victor Snieckus at Queen's or K.C. Nicolaou at Scripps.

Subject: Visit
From: "John Long" <johnl@GFSChemicals.com>
Date: Wed, 9 Jun 2004 09:57:20 -0400
To: <baderfa@execpc.com>

Alfred -

It was good to see you during my many travels last month. The painting made it back just fine, and you'll be seeing a check shortly. The books will be a separate transaction involving GFS, which I will arrange. Are you putting the larger Grathwol on your web site ? The one with the reflection in the lake ? I'd like Melody to see it some time.

I have on file the following information that I believe applies to the talks we anticipate -

1. Museum - talk # 9. The Rembrandt Research Project and the Collector

2. CAS / ACS section - talk # 1. The History of Aldrich Chemical Co.

3. MUACC/OSU (analytical chemists) - Topic is Loschmidt and the benzene molecule - is this the Detective Story (talk # 3 - Richard Anschutz, Archibald Scott Couper and Josef Loschmidt) ?

By the way, I've interested Bill Szabo in some of our organic technology, and we expect to work with him in developing some business connections in California. The Barry Sharpless Scripps work continues to be a major focus for us.

And I spoke with Frank Wagner at Strem. They are busy, still highly agitated at the developments in the Grubbs Catalyst situation. Can't say as I blame them.

Enjoy your travels and best regards to Isabel. JRL

John R. Long, Ph.D.
Director of Technology
GFS Chemicals, Inc.

Dr. Alfred Bader
924 East Juneau, Suite 622
Milwaukee, Wisconsin 53202
Phone: 414/277-0730
Fax: 414/277-0709

A Chemist Helping Chemists

September 24, 1996

Professor Ned D. Heindel
Department of Chemistry
LeHigh University
Seeley G. Mudd Bldg. 6
Bethlehem, PA 18015

Dear Ned:

Surely you remember how influential you were in guiding Bill Wiswesser to write his Loschmidt article published in the *Aldrichimica Acta*. A copy of your key letter of September 9, 1987 is enclosed.

Bill has been maligned terribly, particularly by Professor G.P. Schiemenz at the University of Kiel. In Schiemenz' slide talks, he shows an open grave marked '*William Wiswesser*' and then says "Wiswesser is dead. Let him rest in peace."

I would like to write a rather detailed paper describing some of Bill's work and refuting the criticism. I have already done so to some extent in two detailed papers that I gave at the Loschmidt Symposium in Vienna in June last year. These papers will be published by Plenum before long.

However, these papers and my brief notes in this month's *Chemistry in Britain* do not really deal with the criticism and that I would like to do.

Do you happen to have a curriculum vita of Bill? You probably had that at the time when he received an honorary doctorate from Lehigh. Also, do you know of any printed appreciations of Bill's work? And do you have the addresses and phone numbers of any of his family members?

With many thanks for your help and best regards, I remain,

Yours sincerely,

AB/cw
Enclosures

Dr. Alfred Bader
924 East Juneau, Suite 622
Milwaukee, Wisconsin 53202
Phone: 414/277-0730
Fax: 414/277-0709

A Chemist Helping Chemists

October 4, 1996

Hofrat Prof. Dr. R. Lewisch
Institut für Experimentalphysik
Technische Universität Wien
Wiedner Hauptstrasse 8-10/131
A-1040 Wien
Austria

Dear Hofrat Professor Dr. Lewisch:

I so appreciate your most helpful letter of September 16th with the very instructive enclosures. Please accept my sincere thanks and pass these on also to Professor Dr. Kirchmayr.

It is clear that there were Jewish students in both divisions of your Institute from the very beginning, and of course most of these came from Hungary, Moravia, Bohemia, and surprisingly, Triest.

Dr. Robert Rosner in Vienna and I are working on an article relating to anti-Semitism among chemists in Vienna in the last century, and we will eventually,of course, send you a copy of this.

With all good wishes to you and Professor Dr. Kirchmayr, I remain,

Yours sincerely,

AB/cw

bc: Dr. Robert Rosner

Dr. Alfred Bader
924 East Juneau, Suite 622
Milwaukee, Wisconsin 53202
Phone: 414/277-0730
Fax: 414/277-0709

A Chemist Helping Chemists

August 15, 1996

Professor Clarisse Habraken
Department of Chemistry
Leiden University
Leiden
The Netherlands

Dear Professor Habraken:

Just a note to tell you that I was delighted to see your name in a letter to this week's *C&E News* written by an old, mutual friend, Professor James Moore. It reminded me of our many happy meetings in Leiden.

Incidentally, I come to Holland, usually one week a year, in November, and this year it will be the week of November 11th. It has occurred to me that you might like me to present a talk in chemical history, for instance, Talk #1 or #3 of the enclosed.

With all good wishes from Isabel and me, I remain,

Yours sincerely,

AB/cw

Enclosure

c: Professor James A. Moore (w/enclosure)

Dr. Alfred Bader
924 East Juneau, Suite 622
Milwaukee, Wisconsin 53202
Phone: 414/277-0730
Fax: 414/277-0709

A Chemist Helping Chemists

August 1, 1996

Mr. David P. Leising
211 North Grove Street
Lowell, MI 49331-1406

Dear Mr. Leising:

I am sorry that a long trip to Europe has delayed my responding to your interesting letter of May 28th.

Dr. Alfred Bader, the author of the paper to which you refer, is a very distant relative and a good friend who lives near Lausanne in Switzerland. by a singular coincidence, his father was also called Alfred, as was my father!

I would be happy to have the book you mentioned and am sending you some reproductions in exchange.

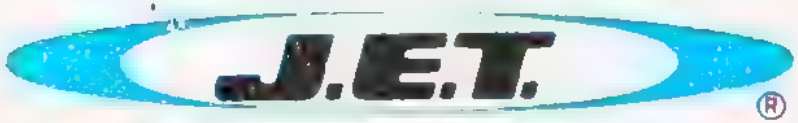
With all good wishes, I remain,

Yours sincerely,

AB/cw

Enclosures

bc: Dr. Alfred Bader (w/enclosure)



Jet Electronics and Technology, Inc

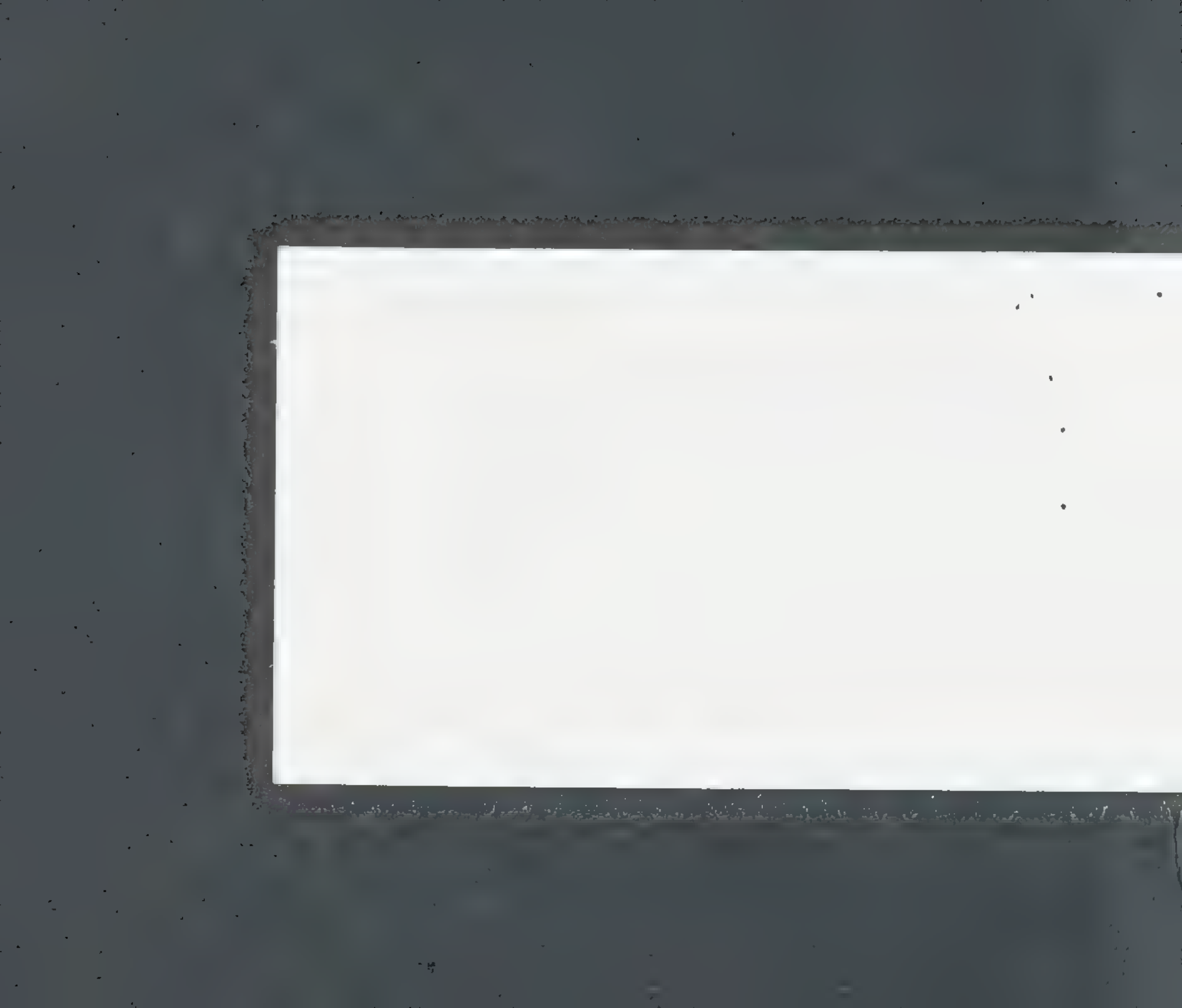
A Subsidiary of The BFGoodrich Company

RETIRED

Dave Leising
Engineer
Advanced Technology

5353 52nd Street
Grand Rapids, MI
49588-0873

Phone: 616-949-6600
Fax: 616-949-9376
Telex: 22-6453



David P. Leising
211 North Grove Street
Lowell MI 49331-1406
May 28, 1996

ATTN: Dr. Alfred Bader
Aldrich Chemical Company, Inc.
1001 West Saint Paul Avenue
Milwaukee WI 53233

Dear Dr. Bader:

I am a retired engineer after 25 years with JET Electronics & Technology, Inc., now a division of BFGoodrich. Although my primary duties were in electronic design and optics, I did some minor work there in chemistry, and have long had a hobby interest in collecting rare elements. Therefore, I received the Aldrich catalogs at work, and thereby learned of you and of your great interest in art.

Some years ago, at an antique store, I acquired a slipcased art monograph titled *Insania Pingens*, published by the CIBA Pharmaceutical Company (Basle, Switzerland, 1961) and featuring the art works of psychiatric patients, with professional commentary.

While casually looking through the book for the Nth time last night, I suddenly took note of the fact that one of the contributors to the volume was a Dr. Alfred Bader! Could this be you, having switched careers from medicine to chemistry, or perhaps a relative of yours, or is this just a coincidence?

In any event, due to your great passion for art, I would be willing to send this volume to you gratis; as I recall I only paid a dollar for it.

Respectfully,

David P. Leising

David P. Leising

David P. Leising
211 North Grove Street
Lowell MI 49331-1406

ATTN: Dr. Alfred Bader
Aldrich Chemical Company, Inc.
1001 West Saint Paul Avenue
Milwaukee WI 53233

53233-2641 50

Dr. Alfred Bader
2961 North Shepard Avenue
Milwaukee, Wisconsin 53211

A Chemist Helping Chemists

July 29, 1996

Professor Pierre Laszlo
Institut de Chimie
Université de Liège
Sart-Tilman par 4000
Liège 1, Belgique

Dear Professor Laszlo:

I am sorry that a long trip to Europe has delayed my responding to your letter of May 31st.

When Isabel saw the Kauffmanns' review of my book in *Angewandte Chemie*, she commented that the review was so good, and also so long, that hardly anyone would order the book: All the important information is already in the review.

The worry about Bob Woodward's papers deteriorating has been around for a long time. As you know, I raised the funds for the Woodward Symposium in Philadelphia, and at the time, told Crystal Woodward that if she and Harvard could raise the funds required, I would gladly contribute $2,000.

I am busier now than ever before and am afraid that I could not take the lead in raising the total amount needed, but frankly, I feel strongly that this should be Harvard's responsibility.

With all good wishes, I remain,

Yours sincerely,

AB/cw

Professeur PIERRE LASZLO

Institut de Chimie
Université de Liège
Sart-Tilman par 4000 Liège 1, Belgique

Dr. Alfred Bader
924 East Juneau
Suite 622
Milwaukee WI 53202
USA

May 31 1996

Dear Dr. Bader,

I've ordered your book from England and I'm looking forward to reading it. Have you seen its review by George Kaufmann and his wife in the latest issue of *Angewandte Chemie*?

The reason for this letter is that I was alerted by Ms. Crystal Woodward of the likely deterioration of her father's manuscripts, dealing with creative organic synthesis and his intellectual tools, that her family had donated to Harvard University. You will find enclosed copy of the correspondence that ensued from a letter that I wrote to President Rudenstine, jointly with my colleague Nguyen Trong Anh from the Ecole polytechnique.

My understanding is that the expense envisaged is relatively modest, ca. $ 25,000. May I suggest that you look into this matter and that , with all your contacts, you take the lead in raising the money needed to ensure preservation of these documents of obvious interest, both to the present chemical community and to future historians?

Do share with your wife my fond regards,

Cordially yours,

Pierre Laszlo

PL:ls
Encls.

De la part de

Pierre Lasz

de l'Université de Liège

HARVARD UNIVERSITY ARCHIVES
CAMBRIDGE, MASSACHUSETTS 02138

PUSEY LIBRARY
(617) 495-2461

15 May 1996

Professor Pierre Laszlo
Professor Nguyen Trong Anh
Département de Chimie
Ecole Polytechnique
91128 Palaiseau Cédex
France

Dear Professors Laszlo and Anh:

Your letter of April 9, addressed to President Rudenstine, has been forwarded to this office for reply. I am sorry for the delay in responding to your inquiry and your quite gratifying endorsement of the importance of the papers of the late Professor Robert B. Woodward. We share your concern for the long term preservation of this valued resource for the history of modern organic chemistry. To date the Harvard Archives has invested considerable time and materials in organizing, and refolding and reboxing the collection in acid neutral materials. I am afraid, however, that the additional and desirable step—to treat selected portions of the collection by mass deacidification—has so far not been feasible. The Archives does not have funds in its budget to undertake this work, although we have been authorized by the director of the University Library to establish a special financial account into which contributions for the work can be placed. Professor Woodward's daughter, Crystal Woodward, has been concerned and active for some time in trying to attract contributions to the fund, but regrettably, to date, none have been received. The project to deacidify the collection has been further complicated by the fact that previously available facilities for such work in the United States have collapsed, due apparently to lack of sufficient support and interest from among the nation's libraries. The University Library's preservation program is currently investigating other facilities for mass deacidification (in the U.S. and in Germany), but no definitive outcome has resulted to date from these queries.

I assure you that we fully appreciate the value of Professor Woodward's papers and your support for their preservation is very much appreciated. We shall

continue to hope for a solution to the problem of their long-term preservation. In the meantime, they are maintained in our best available facilities and there is hope that their further deterioration is at least somewhat slowed. You should know, as well, that a good portion of the collection is on paper of quite respectable quality and poses no real concern for the time being.

Sincerely,

Clark A. Elliott

Clark A. Elliott,
Associate Curator for Archives
Administration and Research
Harvard University Archives

xc: President Neil Rudenstine

Dr. C.A. Elliott
Associate Curator for Archives
Harvard University Archives
Cambridge MA 02138
USA

May 31 1996

Dear Dr. Elliott,

Thank you for your letter of May 15. I'm dismayed that Harvard University would expect Professor Woodward's family to bear the cost of deacidifying his papers, surely some of the most valuable items in the Archives that you are looking after. I'm surprised also on technical grounds, I have a faint recollection of a titanium dioxide process commissioned from a Canadian company by the Library of Congress. If indeed no efficient process is available at present, we ought to alert the chemical community in order to devise solutions to this technical problem.

Cordially yours,

Pierre Laszlo

PL:ls

Dr. Alfred Bader
924 East Juneau, Suite 622
Milwaukee, Wisconsin 53202
Phone: 414/277-0730
Fax: 414/277-0709

A Chemist Helping Chemists

June 20, 1996

Professor Pierre Laszlo
Institut de Chimie
Université de Liège
Sart-Tilman par 4000
Liège 1, Belgique
France

Dear Professor Laszlo:

Thank you for your letter of May 31st to Dr. Bader.

He and Mrs. Bader are in England through the end of July. He will reply personally upon his return to Milwaukee.

Best wishes,

Cheryl Weiss
Office Manager

Dr. Alfred Bader
924 East Juneau, Suite 622
Milwaukee, Wisconsin 53202
Phone: 414/277-0730
Fax: 414/277-0709

A Chemist Helping Chemists

April 29, 1996

Dr. Clinton F. Lane
4854 North Larkin Street
Whitefish Bay, WI 53217

Dear Clint:

It was great being able to spend a couple of days with you, and I hope that we can do that again on the weekend of April 5, 1997.

I was very impressed by Dr. P.V. Ramachandran, who has been with Herbert these last 12 years.

Not surprisingly, he is looking for an academic job, but may fail. If you could get him to Aldrich to handle a small group making chiral compounds by Herbert's patented technology, I think you would find this very productive indeed.

Please thank your wife for her thoughtfulness in wondering whether you should sign the proxy card. But, Clint, the company is far better off with your signing this all in the affirmative and staying in a very responsible position with the company. Tom is one of the most vindictive persons I know, and if it came to his attention that you disagreed, then I would fear for the worst.

With all good wishes, I remain,

Yours sincerely,

AB/cw

bc: H C Brown

March 10, 1995

Mr. Steven L. Ludmerer
299 Tom Brown Road
Moorestown, NJ 08057

Dear Steven:

Thank you so much for your most impressive letter of March 1st.

Recently, Sigma dismissed the head of its diagnostics division, a man by the name of Art Caprio, because of clear ethical improprieties and conflicts of interest.

It seems to me that you might well fit into Sigma's diagnostic division, if not as its top man, then nonetheless, one of its key executives.

Hence, I would suggest that you contact Dr. Tom Cori, the CEO of Sigma-Aldrich in St. Louis.

However, it would be very important that you don't mention in any way that I suggested that. Tom dismissed me under most curious circumstances three years ago and to mention my name would be the kiss of death.

With all good wishes, I remain,

Yours sincerely,

STEVEN L. LUDMERER
299 Tom Brown Road
Moorestown, NJ 08057

609-273-9895 home
215-972-7289 office

March 1, 1995

Dr. Alfred Bader
Astor Suite 622
924 East Juneau Avenue
Milwaukee, WI 53202

Dear Al:

It was a pleasure meeting you and Isabel during the INFORMEX '95 Conference in Nashville. I appreciated your comments and look forward to your upcoming book.

As with you, my entrepreneurial drive is widespread and enduring. After twenty-five years at DuPont and Union Carbide, I joined a biotechnology company in 1992 as the CEO of its diagnostics unit. As I grew the business, it became non-strategic and was sold last year. I am now looking for a new opportunity to build a business and feel that your consulting experience with emerging companies and your knowledge of the industry could help me in my search.

More specifically, my credentials include a variety of marketing, business development and general management in diversified chemical, material, biotechnology and consumer products businesses. My ability to create teams and build businesses has been proven in both the large corporate cultures of companies such as DuPont and Union Carbide, as well as in smaller entrepreneurial organizations. I played key roles in the commercialization of *Teflon*® and other industrial coatings, *Reach*® toothbrush, *Barricade*® protective apparel, the *Quantix*® environmental workstation and a wide variety of other industrial products. I built two businesses from scratch and have led operations with $50MM revenue.

At this time I am interested in identifying and building a business unit where novel technology coupled with my skills can bring a step-change in performance to customers. I am well qualified to address challenges in both consumer and industrial businesses. I also bring a keen understanding of international business issues through my resident experience in Asia and global business responsibilities.

I'd appreciate your assistance with suggestions on how I might expand my search for new opportunities. To that end, I'll give you a call next week. Please understand that I recognize that it is unlikely you will know of a suitable opportunity at this time. Nevertheless, I would like to share my search process and get any ideas or suggestions you might have.

Sincerely,

Steven L. Ludmerer

STEVEN L. LUDMERER

299 Tom Brown Road
Moorestown, NJ 08057
609-273-9895

OBJECTIVE

General management executive position where my leadership, strategic analysis, and innovation expertise can provide profitable growth to a company.

BACKGROUND SUMMARY

Domestic and international experience at progressive levels of business management, marketing management, strategic business planning, and research and development. Industry experience spans chemicals, fibers, biotechnology, consumer package goods and service industries. Built businesses from concept to multi-million dollar volume. Responsibilities included: P/L accountability; development and commercialization of high technology products; and acquisitions and divestitures.

PROFESSIONAL EMPLOYMENT

QUANTIX SYSTEMS (*Subsidiary, DNA Plant Technology Corporation*) **1992 - 1994**

President and Chief Executive Officer

Reported to CEO of parent company. Quantix developed, manufactured and marketed rapid on-site environmental analysis systems using immunoassay technology.

- Redirected business from agricultural to environmental markets, growing environmental revenues from zero in 1991 to $2MM in 1994.
- Introduced six new products to analyze for petroleum and pesticide contamination.
- Negotiated five year requirements contract with DuPont yielding $3MM in revenue.
- Acquired exclusive, preemptive patent rights to several products and technologies from Monsanto.
- Enhanced employee dedication during redirection and later negotiations for sale of the business.
- Member of policy committee which raised $20MM equity for NASDAQ listed parent company.

UNION CARBIDE CHEMICALS AND PLASTICS COMPANY **1989 - 1992**

Business Director - Specialty Chemicals

Reported to Vice President. Charter was to establish one billion dollar division in high margin growth businesses with a ten year $350MM investment commitment.

- Led investment portfolio in environmental products and services, biomaterials and health care with $6MM budget.
- Shutdown, sold, or redirected operations in recycling, consulting, and sample packaging, saving $8MM.
- Developed and introduced *UCAIR™* - air cleaning system for indoor air pollution abatement.

E.I. DUPONT DE NEMOURS AND COMPANY **1965 - 1989**

Manager - Safety and Environmental Services 1987 - 1989

Established and grew environmental management consulting, training and seminar business from conception in 1987 to $3MM in 1988. Later gained domestic and international responsibility for marketing of safety consulting, seminar and training materials. Total revenue $30MM, 35 people.

- Full P/L responsibility. Increased environmental staff from three to twenty. Built dedicated team.
- Introduced new consulting services, seminars, video, interactive video, and software.

E.I. DUPONT DE NEMOURS AND COMPANY (continued)

Manager - Development Businesses 1984 - 1987

Managed major new growth opportunities including: specialty polymers, composites, environmental management, and biomedical markets.

- Identified need for protection of personnel in hazardous environments, sponsored successful research to identify polymer/fabric laminates and evaluated opportunity to market roll goods vs. branded apparel. Result was *Barricade*™- a $30MM brand.

Worldwide Product and Marketing Manager 1982 - 1984

Directed marketing, sales, product planning and technical aspects of $50MM industrial finishes business.

- Implemented restructuring plan merging compatible operations and divesting others to Mobil and Whittaker.

Regional Director - Business Development 1979 - 1982

Reported to President, DuPont Asia-Pacific, a $1.5 billion business. Responsible for overall corporate development and investment strategy for the region from a base in Hong Kong.

- Established manufacturing operations, negotiated distribution and joint venture agreements, and obtained government incentives for agrichemicals, pigments, polymers, fibers, electronics, and medical products businesses in region from Japan to New Zealand to Pakistan.

Product Manager - Packaging Materials 1977 - 1979

Led marketing and technical activities for $20MM business in coatings for cans and paper, film, foil laminations.

- Secured first two $1MM sales contracts for this business.

Division Planning Manager 1975 - 1977

Negotiated and implemented divestiture of consumer products businesses, including *Reach*® toothbrush.

- Successfully sold manufacturing plant providing continued employment for 200 people.

Manager - New Consumer Businesses 1974 - 1975

Directed development and acquisition of technology and products for car care and toiletries product lines.

- Developed *Reach*® toothbrush, *Good 'n Clean*® hand cleaning lotion, *Carpet Fresh*™- others.

Corporate Planning Consultant 1972 - 1973

Worked for executive committee to develop corporate information system and manage capital investments.

Research and Development Positions 1965 - 1972

Key developer of *Teflon II*® coatings for cookware. Supervised 25 employees in industrial coatings research.

EDUCATION

MBA, DREXEL UNIVERSITY, Philadelphia, PA 1971

BS, Chemical Engineering, NORTHEASTERN UNIVERSITY, Boston, MA 1965

NORTHWESTERN UNIVERSITY, Kellogg School, Evanston, IL 1993
Merger Week, Alfred Rappaport Seminar

UNIVERSITY OF VIRGINIA, Darden School, Charlottesville, VA 1987
Business Executive Program

ALFRED BADER FINE ARTS

DR. ALFRED BADER

ESTABLISHED 1961

July 31, 1995

Dr. Peter Pollak
Vice President/General Manager
Special Fine Chemicals
Lonza, Inc.
17-17 Route 208
Fair Lawn, NJ 07410-2821

Dear Peter:

I have just returned from a long trip to Europe and found your kind note of June 7th advising that you will be working in Fair Lawn for awhile.

Is there a chance that you might come and visit Aldrich in Milwaukee, and of course, also me?

I hope that you will like what I have written about you in *Adventures of a Chemist Collector*, review enclosed.

Best personal regards, as always,

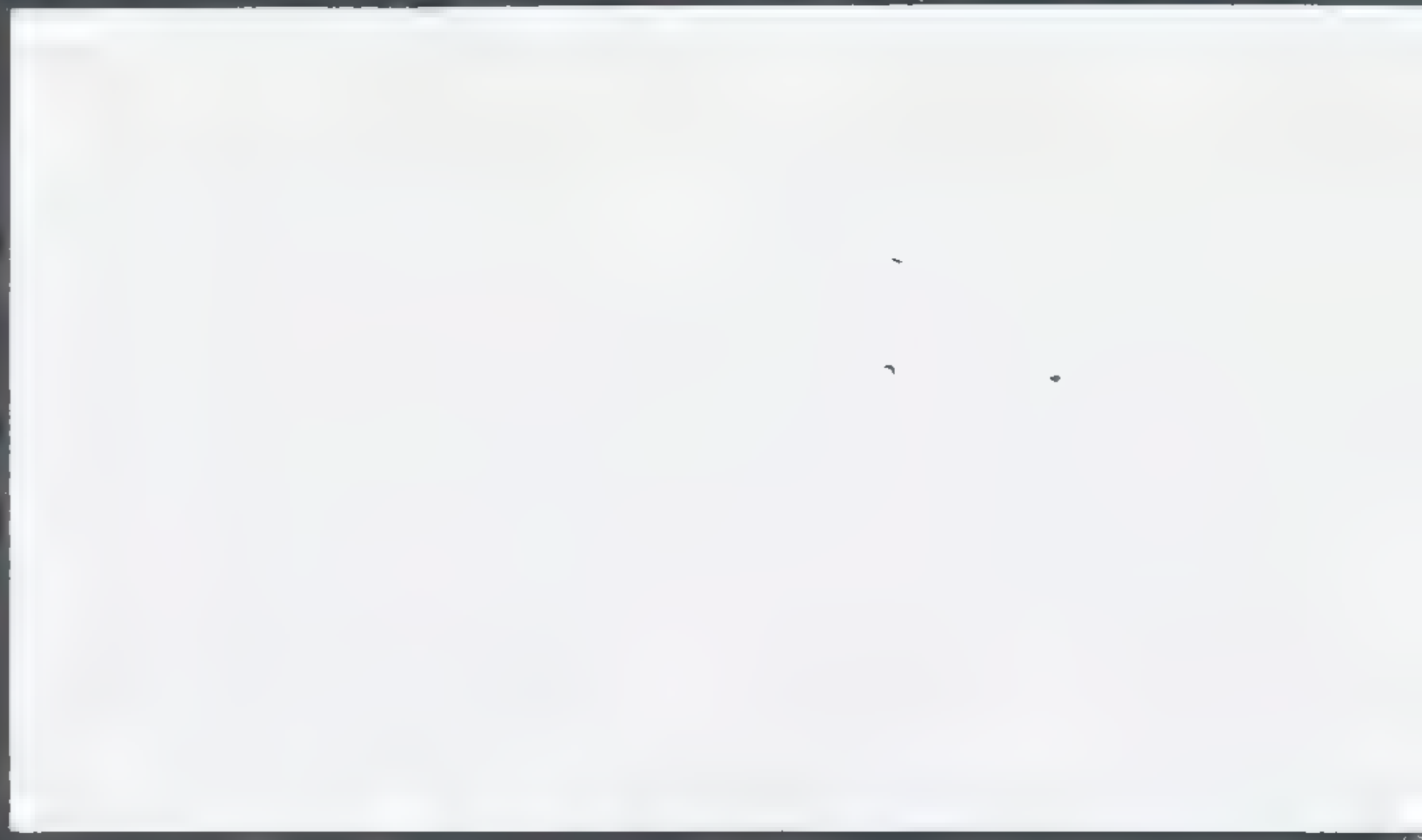
AB/cw

By Appointment Only
ASTOR HOTEL SUITE 622
924 EAST JUNEAU AVENUE
MILWAUKEE WISCONSIN USA 53202
Tel 414 277-0730 Fax 414 277-0709

LONZA

DR. PETER POLLAK

Vice President / General Manage



LONZA AG Basel, Schweiz
LONZA SA Bâle, Suisse
LONZA LTD Basel, Switzerland

Feinchemikalien

Münchensteinerstrasse 38
CH–4002 Basel
Telefon 061 316 81 11
Fax 061 316 83 01
Telex 965 960 41 lon ch

LONZA

Dr.
Alfred Bader
2961 North Shepard Avenue
USA Milwaukee, WI 53211

Ihre Referenz
Unsere Referenz FSF PP/jst
Durchwahl 061 / 316 83 47
Basel, 7. Juni 1995

Lieber Alfred,

Zur Verstärkung unserer Marktpräsenz in den USA werde ich bis zum Jahresende bei unserer amerikanischen Tochtergesellschaft LONZA Inc., Fair Lawn, tätig ein. Dabei werde ich die weltweite Marketing-Verantwortung für das Spezial Feinchemikalien-Geschäft behalten. Für die tatkräftige Fortführung der Geschäfte ausserhalb USA werden meine langjährigen Mitarbeiter Marc New (Pharma) und Gérard Samuel (Agro) mit ihren Teams besorgt sein.

Mit freundlichen Grüssen

Dr. Alfred Bader
2961 North Shepard Avenue
Milwaukee, Wisconsin 53211

A Chemist Helping Chemists

September 29, 1995

Dr. John Long
4301 Copper Ridge Road
Champaign, IL 61821

Dear John:

It was such a pleasure to be able to chat with you this morning.

Enclosed as promised is the 'menu' of my talks. I would love to be invited to your ACS Section and the University to give a few talks.

Also, of course, I do hope that when next you come to Milwaukee, you can spend a little while with us.

With all good wishes from house to house, I remain,

Yours sincerely,

AB/cw

Enclosure

Dr. Alfred Bader
2961 North Shepard Avenue
Milwaukee, Wisconsin 53211

A Chemist Helping Chemists

September 18, 1995

Mr. R. Loomer
675 Highland Avenue
Ottawa, Ontario K2A 2K5
Canada

Dear Mr. Loomer:

Thank you for your two notes, the second explaining about the book.

Isabel and I have heard nothing but good things about the Elderhostel programs, but just don't know whether Herstmonceux is considering participating. Why not ask the International Student Centre, Richardson Hall, Queen's University, which administers the programs at the Castle?

With all good wishes, I remain,

Yours sincerely,

AB/cw

Aug 31, 1945

Dear Dr. Fosdick,

What a pleasant surprise to receive your book, thank you very much. I look forward to reading about your life. It will be a pleasure & opportunity to correspond on

the open questions with Elizabeth. My husband and I would be glad to stay on in Edinburgh for some time yet. But as an international project of education in view of the condition of the world, I cannot see any future for Pendle Hill, as a meeting together with all sorts and conditions of human humanity with one heart on Edinburgh at the Conference

Calypso
by Allan Fournier

This exclusive card was created by the Canadian Wildlife Federation for people who are concerned about wildlife and the environment. Proceeds from its sale will help fund CWF's conservation and education programs

Canadian Wildlife Federation
2740 Queensview Drive
Ottawa, Ontario K2B 1A2

Calypso bulbeux
par Allan Fournier

Cette carte est une création exclusive de la Fédération canadienne de la faune, proposée aux personnes qui ont la nature à cœur. Les profits de sa vente sont destinés à des programmes de conservation de nos richesses naturelles et d'éducation.

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2740, promenade Queensview
Ottawa (Ontario) K2B 1A2

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Air Mail Par a

FAX FROM

DR. ALFRED R. BADER'S OFFICE
Suite 622
924 East Juneau Avenue
Milwaukee, Wisconsin 53202
Telephone: 414/277-0730
Fax: 414/277-0709

July 14, 1995

To: Dr. Nquin Liu
Fax: 011-852-249-17645

Dear Dr. Nquin Liu:

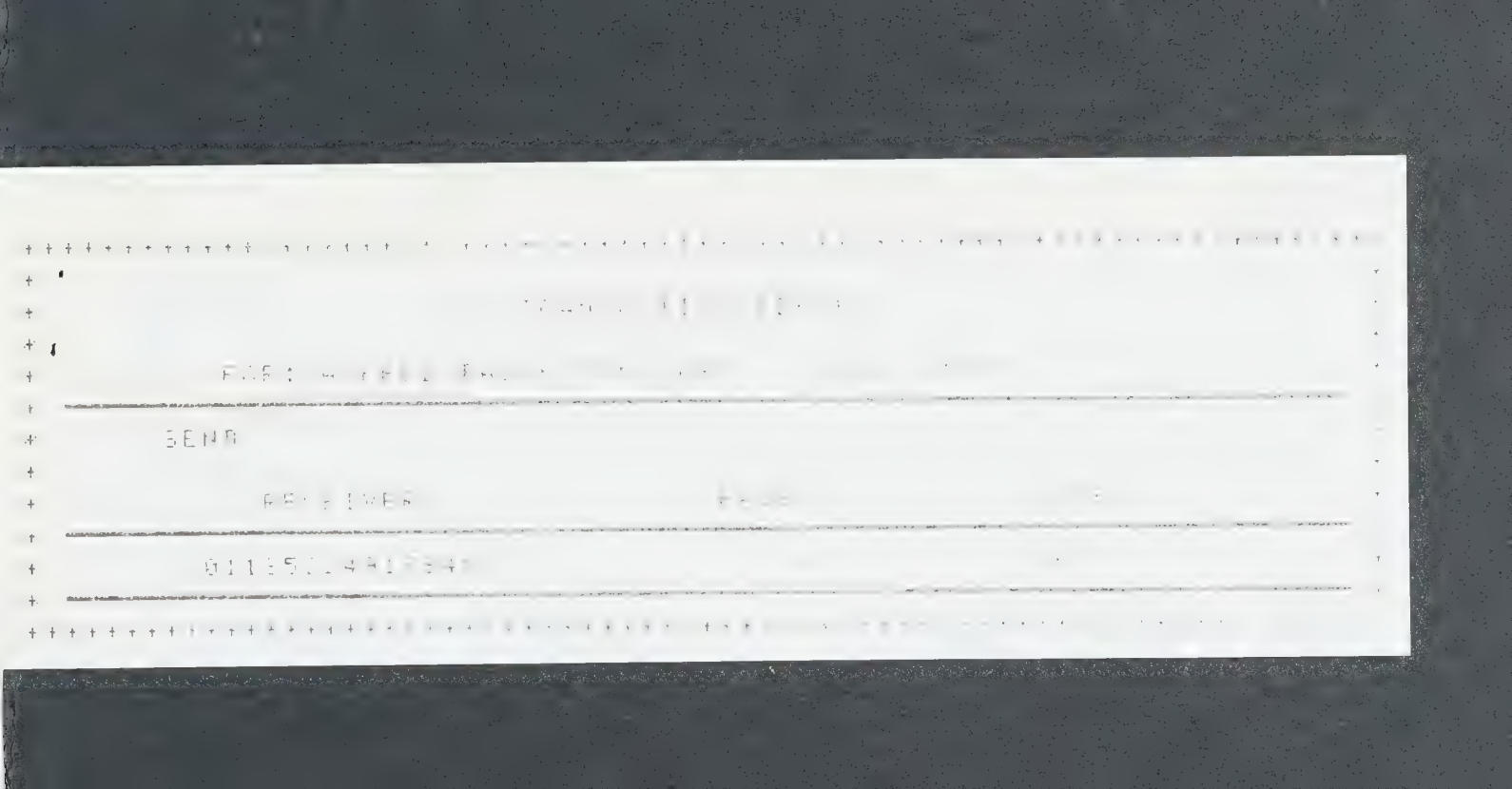
Dr. Bader is presently traveling in England, but he has received your message and confirmed that he will be attending the ACS meeting in Chicago in August and that he will be happy to speak with you there.

If you wish to let Dr. Bader know how to reach you while you are in Chicago and to arrange a mutually convenient time to talk, please fax your travel plans or other information to Dr. Bader's office number as shown above. Thank you.

Best wishes,

Cheryl Weiss

Cheryl Weiss
Office Manager



[illegible]

Dear Albert

A [illegible]

McQuinn [illegible] [illegible] from
Hungary [illegible] the [illegible] [illegible]
at the time of the ACS meeting
of 26 August at Chicago - He will
call you, [illegible] to [illegible] [illegible]
the [illegible] numbers.

FAX [illegible] - [illegible]

Phone [illegible] [illegible]

I would be glad something [illegible] [illegible]
a reagent problem [illegible] [illegible]
discuss with you

[illegible]

[illegible]

Dr. Alfred Bader
924 East Juneau, Suite 622
Milwaukee, Wisconsin 53202
Phone: 414/277-0730
Fax: 414/277-0709

A Chemist Helping Chemists

November 1, 1995

Mr. Irwin Schwartz
Chief Chemist
Jos. H. Lowenstein & Sons
420 Morgan Avenue
Brooklyn, NY 11222

Dear Irwin:

Your fax dated October 6th arrived here on October 18th, while I was on a rather long trip to Canada, from which I have only just returned.

As I told you, I have purchased about 10% of the stock of Anglo United, which bought Coalite some years ago. I plan to visit Anglo United later this month, but I understand from their managing director that by the time of my visit, Coalite and Coalite Chemical might well have been sold.

Of course, they couldn't and wouldn't tell me who the buyer is, but I understand through the grapevine that it is likely to be a fast-expanding South African company, Sasol, described in the enclosed article that you have probably seen. Of course, it makes sense for Sasol to want to purchase Coalite and Coalite Chemical, for all the reasons which are obvious from that article.

Thus, my ownership in Anglo United will probably not help, but of course, I will try and touch base with the buyer and do what I can.

It will be obvious from my book that I think of you often and not only because you gave me one of my favorite ties, Xerox enclosed.

It might be that you will like my autobiography, available from Trafalgar Square, details enclosed. I cannot - by contract - sell single books, but if Lowenstein might like 10 or more copies, for Hanukkah gifts to your best customers, I can supply these at a 50% discount, that is, at $12.50 per book.

Isabel and I are leaving for England on November 13th and will, of course, be in touch with you after we have definite information.

Fond regards, as always,

AB/cw

FAX MESSAGE

jos. h. lowenstein & sons, inc.

PHONE: (718) 388-5410 FAX: (718) 387-3806

DATE

TO

FROM

NUMBER OF PAGES
(including this one)

ATTENTION

Dr. Alfred Bader
2961 North Shepard Avenue
Milwaukee, Wisconsin 53211

November 10, 1993

Dr. John R. Long
932 Fifth Avenue
Grafton, Wisconsin 53024

Dear John,

I am just working on my autobiography and enclose a rough draft of the chapter dealing with inorganics. Could you please check the date when you joined Aldrich, and also point out mistakes that come to your mind.

Many thanks, and all good wishes.

Sincerely,

Enclosure

FAX FROM

DR. ALFRED R. BADER
Suite 622
924 East Juneau Avenue
Milwaukee, Wisconsin 53202
Telephone 414-277-0730
Fax No. 414-277-0709

August 4, 1993

To: Mr. Bert Van Deun
Company Group Chairman
Johnson & Johnson
Fax 908 828 3912

Dear Bert:

Please do not mind that my long trip to Europe has delayed my responding to your important inquiry of June 22nd regarding your requirement for large quantities of p-hydroxyphenethyl alcohol.

It seems to me that this is the very kind of compound which can be made by hydroboration, and so I have taken the liberty of sending your inquiry to Dr. Clinton Lane at Aldrich. He probably knows more about hydroboration than any other industrial chemist, and I am asking him to contact you directly.

Of course we very much hope that you and Mia will visit before we travel again to Europe in the fall.

Fond regards from house to house,

c: Dr. Clinton Lane

6/74

Johnson & Johnson

BERT VAN DEUN
COMPANY GROUP CHAIRMAN

ONE JOHNSON & JOHNSON PLAZA
NEW BRUNSWICK, NJ 08933
TEL. (908) 524-3615
FAX (908) 828-3912

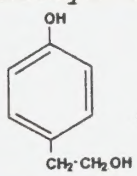
June 22, 1993

Dr. Alfred Bader
Alfred Bader Gallery
Suite 622
924 East Junean Avenue
Milwaukee, WI 53202

Dear Alfred:

Noramco is developing a UV stabilizer, trademark NORBLOC, a patented benzotriazole that offers a unique characteristic: the ability to polymerize.

The size and profitability of this business will be dependent upon our ability to get manufacturing costs down. The main opportunity to do so seems to be to reduce the costs of the main intermediate: p-hydroxy phenethyl alcohol.



We are buying this product right now from the only supplier we know: Otsuka in Japan. We learned, however, that Hoechst France, Hoechst-Celanese and Pyosa (Mexico) might be interested in developing it in commercial quantities.

The purpose of this letter is to ask you whether you know of any other source, or a potential source, because so much depends on it for us. Any advice you might be able to give us would be greatly appreciated.

With fond regards.

Yours ever,

Bert Van Deun

s

FROM THE DESK OF

BERT VAN DEUN

6/22/93

Marilyn:

This can wait until Alfred gets back.

B.