DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
College Park, MD 20740

September 26, 2005

C.K. Gund, Ph.D.
Phoenix Regulatory Associates, Ltd.
21525 Ridgetop Circle, Suite 240
Sterling, VA 20166 USA

Re: Food Contact Substance Notification FCN 000531

Dear Dr. Gund:

This is in reference to your notification for the food contact substance and use described as follows:

Food Contact Substance
Agarose, polymer with (chloromethyl)oxirane, 2-hydroxy-3-(2-hydroxy-3-(trimethylammonio)propoxy)propyl ethers, sulfate salts (CAS Reg. No. 846053-13-2).

Notifier
GE Healthcare

Manufacturer/Supplier
GE Healthcare

Intended Use
As an ion exchange resin.

Limitations/Specifications
For repeated use in extracting individual proteins or substances present in similar low concentrations from liquid, water-based food materials, such as milk, whey, fruit juice, beer and wine.

For continuous use, the process conditions will be between pH 3 and 12 and temperatures below 40°C. Use at pH 12 up to 40°C is limited to a maximum of 2000 hours over the resin lifetime. Cleaning at 20°C may take place at up to pH 14, cleaning at 40°C conducted at up to pH 13.7, and cleaning at 60°C conducted at up to pH 13.3.

This is to inform you that as of October 26, 2005, FCN 000531 will become effective. It will be added to the list of effective notifications for food contact substances available on the agency's internet site at http://vm.cfsan.fda.gov/~dms/opa-fcn.html.

The Agency has determined under 21 CFR 25.32(j) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

This effective notification is applicable only to Agarose, polymer with (chloromethyl)oxirane, 2-hydroxy-3-(2-hydroxy-3-(trimethylammonio)propoxy)propyl ethers, sulfate salts (CAS Reg. No. 846053-13-2) manufactured by GE Healthcare and is limited to the use of the food contact substance identified above. You should inform the agency of any modification in the FCS limitations/specifications given in the notification or of any alteration in the manufacturing process that would result in a change in the impurities in the FCS. Such changes may require submission of a new notification.

The existence of an effective notification for a food contact substance does not relieve use of the subject substance from compliance with any other provision of the Federal Food, Drug, and Cosmetic Act or with 21 CFR §174.5 General provisions applicable to indirect food additives. For example, in accordance with section 402(a)(3) of the Act, use of the food contact substance should not impart odor or taste to food rendering it unfit for human consumption.

If new data or information become available to FDA demonstrating that the intended use of the food contact substance is no longer safe, the agency will inform you of its determination that the intended use of the food contact substance is no longer safe. In addition, if you become aware of data that raise questions about the safety of the intended use of the food contact substance, you should notify the agency immediately and be prepared to supply data necessary to resolve the questions.

If you have any further questions concerning this matter, please do not hesitate to contact us.

Sincerely,

Elizabeth R. Sánchez, Ph.D.
Division of Food Contact Notifications
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration

VERSUCHS- UND LEHRANSTALT FÜR BRAUEREI IN BERLIN

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Versuchs- und Lehranstalt für Brauerei in Berlin (VLB) · Seestrasse 13 · D–13353 Berlin
im Institut für Gärungsgewerbe und Biotechnologie

INTERMAG GmbH
Gesellschaft für Brautechnik
Herrn Michael Katzke
Hilgestraße 20

D-55294 Bodenheim

Gutachten über die Löslichkeit des Stabilisierungsmittels "Intergarant® Kombi" (Intermag GmbH, Bodenheim)

0 Einleitung

Die geltende Bierverordnung vom 2. Juli 1990 (BGBl, Jg. 1990, Teil 1, S. 1332-1333), zuletzt geändert am 23. November 1993 (BGBl, Jg. 1993, Teil 1, S. 1912), besagt, daß gewerbsmäßig unter der Bezeichnung "Bier" nur Getränke in den Verkehr gebracht werden dürfen, die gegoren sind und den Vorschriften des Vorläufigen Biergesetzes § 9, Abs. 1,2,4 bis 6 und 11 und den §§ 16 bis 19, §20 Abs. 1 Satz 2 und §§21 und 22 Abs.1 der Verordnung zur Durchführung des Vorläufigen Biergesetzes und §4 Abs. 1 u. 2 der neuen Verordnung entsprechen.

Nach diesen Vorschriften (§ 9 Abs. 6) dürfen als Klärmittel nur solche verwendet werden, die mechanisch oder adsorbierend wirken und bis auf gesundheitlich, geruchlich und geschmacklich unbedenkliche, technisch unvermeidbare Anteile wieder ausgeschieden werden.

1 Stabilisierungsmittel "Intergarant® Kombi"

Bei dem Produkt "Intergarant® Kombi" handelt es sich um einen stark anionischen Ionenaustauscher, der, unter der Bezeichnung "Q Sepharose® Big Beads" von der Firma Pharmacia Biotech AB, Uppsala, Schweden, entwickelt, hauptsächlich für industrielle Anwendungen im biomedizinischen Bereich konzipiert ist. Das Austauschmedium besteht aus 100-300 µm großen Partikeln von hoher physikalischer Stabilität. Die Ionenaustauschgruppen sind an eine hochvernetze Agarose-Matrix gebunden und haben über fast den gesamten pH-Bereich eine hohe Bindungskapazität für Eiweißmoleküle. Dies träfe auch die Bedingungen im Bier (pH-Bereich ca. 4,0 bis 5,0), wo die adsorptive Entfernung von Eiweiß einen Beitrag zur nichtbiolgischen Haltbarkeit des Bieres leistet.

Die Regenerierung des Mittels erfolgt für ionisch gebundene Proteine mittels 2 m Natriumchloridlösung, die von hydrophob gebundenen Proteinen oder Lipoproteinen mit 1 m Natriumhydroxydlösung.

Eine andere als die protein-adsorbierende Eigenschaft der Q Sepharose unter den Bedingungen des Bieres bzw. des Braubetriebes ist nicht erwartet worden, dennoch wurden geprüft, ob durch den Einsatz von "Intergarant® Kombi" bei der Bierstabilisierung sowie durch Reinigungs- und Konditionierungsmaßnahmen Inhaltstoffe im Bier gelöst wurden.

Das Produkt wird aus hygienischen Gründen unter einer 20%igen (v/v) Ethanollösung gelagert und geliefert.

2 Mögliche lösliche Stoffe von "Intergarant® Kombi"

Im Pharmacia "Regulatory Support File: Q Sepharose Big Beads" [1], Section 7, sind die unter Extrembedingungen extrahierbaren Bestandteile von Q Sepharose genannt, die teils aus dem Produkt selbst oder als Rückstände aus dem Herstellungsprozeß stammen können.

Davon wurden ausgewählt:

1 Toluol

2 Epichlorhydrin

3 Allylglycidylether

4 Aceton

5 Ethanol

6 Glucose

7 Hydroxymethylfurfural

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[1] Regulatory Support File Q Sepharose Big Beads, Vers. No 1.2 v. 20.02.95, Pharmacia Biotech, Uppsala, Schweden

3 Versuchsaufbau

3.1 Analytik

Die Analytik wurde z.T. dem "Support File" [1)] entnommen, so die Bestimmung der flüchtigen Bestandteile (1-5) aus Section 9 part 5:

A)

Bestimmung von Toluol, Epichlorhydrin, Allylglycidylether, Aceton, Ethanol mittels Headspace-Gaschromatographie:

Gaschromatograph	HEWLETT PACKARD 5890
Säule	30 m * 0,25 mm I.D. Kapillarsäule, DB-WAX und FFAP
Ofen	Programm 80 °C, 0,5 min, Heizrate 15 °C/min, 200 °C ,1 min,
Injektor	210 °C,
Trägergas	Wasserstoff ca.1 ml/min
	Splitverhältnis 1 : 66,
Detektor	220 °C,
Flammenionisations-Detektor (FID)	
Brenngase	Wasserstoff, 18 psi,
	synth. Luft, 28 psi
Make up-Gas	Stickstoff, 48 psi
Injektion	10 ml Headspace
Integrator	Hewlett Packard

B)

Die Bestimmung eventuell abgegebener Glucose wurde nach MEBAK ausgeführt:

Kohlenhydrate nach Hydrolyse und enzymatische Glucosebestimmung, Hexokinase-Methode [2)]:

Hydrolytische Spaltung von Glucosacchariden mit 25%iger HCl

Enzymatische Bestimmung der Glucose mittels Test Combination Glucose, Boehringer, Mannheim.

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2) Mitteleuropäische Brautechnische Analysenkommission (MEBAK), H. Pfenninger (Hg.), Freising-Weihenstephan: Selbstverlag der MEBAK, Bd II, Kap 2.38.2, S. 246 ff.

C)

Hydroxymethylfurfural wurde nach einer Arbeitsvorschrift von Wackerbaueret al. [3] ausgeführt:

Extraktion von HMF aus Bier mittels Ethylacetat, Einengung zur Trockene, Aufnehmen im Elutionsmittel.

Hochleistungs-Flüssigkeitschromatographie

HPLC	Merck-Hitachi
Injector	Rheodyne 7125 (20 µl)
Säule	250 mm Nucleosil 5 C_{18}
Elutionsmittel	Acetonitril:Wasser 15:85 v/v
Fließgeschwindigkeit	1 $mlmin^{-1}$
Detektor	UV 277 nm
Integrator	Merck-Hitachi

3.2 Versuchsaufbau

Die Stabilisierungseinrichtung wurde entsprechend Versuchaufbau der Fa. "Intermag" zusammengestellt, mit der Ausnahme, daß die Flüssigkeiten Wasser, Bier, NaCl-Lösung und NaOH-Lösung mittels Treibgas (CO_2) gefördert wurden. Bier wurde aus dem Keg mittels Fitting, die anderen Flüssigkeiten aus einem AfG-Container entnommen.

Die Versuche im einzelnen:

Beladen der Säule möglichst luftfrei entsprechend "Intermag"-Vorschrift [4].

Unbehandeltes, nicht stabilisiertes Bier, P(robe) 0

Stabilisierungsprogramm:
Leerdrücken der Säule mit 250 ml Wasser, P 1
100 ml NaCl-Lösung
250 ml Wasser
250 ml NaOH-Lösung
250 ml Wasser, P2
1. Bierlauf mit 8 l/h über 6 Stunden, Temperatur 0 - 1 °C
Bierlauf nach 1 h, P3
Bierlauf nach 2 h, P4
Bierlauf nach 3 h, P5

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[3] Wackerbauer, K., Krämer, P, Methner,. F.-J., Marx, U., Mschr. Brauwiss. 36 (11), 439-442, 1983

[4] Intergarant® Kombi, Datenblatt , Fa. Intermag GmbH, Hilgestraße 20, 55294 Bodenheim/Rh.

Bierlauf nach 4 h, P6
Bierlauf nach 5 h, P7
Bierlauf nach 6 h, P8
250 ml Wasser, P9
500 ml NaCl-Lösung, entgegengesetzte Fließrichtung
250 ml Wasser
1.600 ml NaOH-Lösung (40 °C)
500 ml Wasser, P10
Verschlossene Säule 14 Tage aufbewahrt

Anfahrprogramm wie oben
250 ml Wasser vor Bierlauf, P11
2. Bierlauf mit 8 l/h über 6 Stunden, Temperatur 0-1 °C
Bierlauf zu Beginn, P12
Bierlauf nach 1 h, P13
Bierlauf nach 2 h, P14
Bierlauf nach 3 h, P15
Bierlauf nach 4 h, P16
Bierlauf nach 5 h, P17
Bierlauf nach 6 h, P18
250 ml Wasser, P19
500 ml NaCl-Lösung, entgegengesetzte Fließrichtung
250 ml Wasser, P20
1.600 ml NaOH-Lösung (40 °C), P21
500 ml Wasser, P22

Zum Vergleich (zu P0 bis P22)
mit Kieselgel stabilisiertes Bier, P23

Langzeitversuche:
Exposition einer entalkoholisierten "Intergarant® Kombi"-Suspension in einer Bierprobe:
unbehandeltes Bier, 7 Tage bei Raumtemperatur 22 °C, pH 4,51, P24
mit 60 mg "Intergarant® Kombi behandeltes Bier, entspr. 12 g/hl in 500 ml, 7 Tage bei Raumtemperatur 22 °C, pH 4,51, P25
mit 60 mg "Intergarant® Kombi behandeltes Bier, entspr. 12 g/hl in 500 ml, 7 Tage bei Raumtemperatur 22 °C, auf pH 4,00 eingestellt, P26
mit 60 mg "Intergarant® Kombi behandeltes Bier, entspr. 12 g/hl in 500 ml, 7 Tage bei Raumtemperatur 22 °C, auf pH 5,00 eingestellt, P27

Exposition einer entalkoholisierten "Intergarant® Kombi"-Suspension in einer Probe anderen Bieres: unbehandeltes Bier, 7 Tage bei Raumtemperatur 22 °C, P28
mit 60 mg "Intergarant® Kombi behandeltes Bier, entspr. 12 g/hl in 500 ml, 7 Tage bei Raumtemperatur 22 °C, P29

4 Ergebnisse

Versuchsbier (Faßbier Pilsener Typ)

Stammwürze: 11,04 Gew-%
Scheinbarer Extrakt: 2,64 Gew-%
Wirklicher Extrakt: 4,24 Gew-%
Alkohol: 3,49 Gew-% (= 4,45 Vol-%)
Vergärungsgrad: 76,9 %
pH-Wert: 4,51

Probe	Aceton ppm	Toluol [5] ppm	Epichlorhydrin [5] ppm	Allylglycidylether ppm	Ethanol [6] ppm	Glucose [7] g/100 ml	HMF ppm
P0	n.n.	n.n.	n.n.	n.n.	n.n.	2,72	1,7
P1	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
P2	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
P3	n.n.	n.n.	n.n.	n.n.	n.n.	2,81	1,7
P4	n.n.	n.n.	n.n.	n.n.	n.n.	3,24	1,8
P5	n.n.	n.n.	n.n.	n.n.	n.n.	2,68	1,8
P6	n.n.	n.n.	n.n.	n.n.	n.n.	3,15	1,7
P7	n.n.	n.n.	n.n.	n.n.	n.n.	2,94	1,4
P8	n.n.	n.n.	n.n.	n.n.	n.n.	2,98	1,3
P9	n.n.	n.n.	n.n.	n.n.	n.n.	0,05	n.n.
P10	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
P11	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
P12	n.n.	n.n.	n.n.	n.n.	n.n.	2,81	1,3
P13	n.n.	n.n.	n.n.	n.n.	n.n.	2,89	1,3
P14	n.n.	n.n.	n.n.	n.n.	n.n.	2,68	1,5
P15	n.n.	n.n.	n.n.	n.n.	n.n.	2,76	1,3
P16	n.n.	n.n.	n.n.	n.n.	n.n.	2,81	1,4
P17	n.n.	n.n.	n.n.	n.n.	n.n.	2,94	1,3
P18	n.n.	n.n.	n.n.	n.n.	n.n.	3,11	1,6
P19	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
P20	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
P21	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
P22	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.

n.n. unter Versuchsbedingungen nicht nachweisbar.

5) Toluol, Epichlorhydrin: überlappende Peaks mit denen von anderen, nicht identifizierten Bierinhaltsstoffen auf DB-WAX-Säule, Trennung auf Säule anderer Polarität.

6) Keine signifikante Ethanolzunahme bei 4,45 vol-% im Bier (=44.500 ppm + x) festgestellt.

7) Gesamtglucose nach Hydrolyse aller im Bier vorhandenen Stärkereste (Dextrine)

Probe	Aceton ppm	Toluol [5] ppm	Epichlorhydrin [5] ppm	Allylglycidylether ppm	Ethanol [6] ppm	Glucose [7] g/100 ml	HMF ppm
P23	n.n.	n.n.	n.n.	n.n.	n.n.	2,89	1,5
P24	n.n.	n.n.	n.n.	n.n.	n.n.	2,90	2,0
P25	n.n.	n.n.	n.n.	n.n.	n.n.	2,91	1,9
P26	n.n.	n.n.	n.n.	n.n.	n.n.	2,92	1,9
P27	n.n.	n.n.	n.n.	n.n.	n.n.	2,89	1,9
P28	n.n.	n.n.	n.n.	n.n.	n.n.	3,33	5,5
P29	n.n.	n.n.	n.n.	n.n.	n.n.	3,63	5,9

n.n. unter Versuchsbedingungen nicht nachweisbar.

5 Schlußfolgerungen

Die Verwendung von "Intergarant® Kombi" der Fa Intermag GmbH, Bodenheim, unter üblichen Bierstabilisierungsbedingungen (Bier-pH, Temperatur) wie unter Extrembedingungen (pH, Temperatur, Einwirkzeit) zeigte bei der anschließenden Untersuchung der Testbiere

- keine Detektion bzw. keine Zunahme flüchtiger Substanzen;
- bei Schwankungen (Analysenungenauigkeit, Versuchsfehler) im Bereich 0,2 - 0,3 % keinen signifikante Zusammenhang zwischen dem gemessenen Glucosegehalt und der Bierklärung mit Intergarant® Kombi;
- unter Berücksichtigung der Analysenungenauigkeit keinen Einfluß auf den HMF-Gehalt durch die Bierklärung.

Allgemein konnte eine Löslichkeit des Klärmittels in Bier nicht festgestellt werden. Daraus wird abgeleitet, daß das Produkt "Intergarant® Kombi" den o.g. Anforderungen der Bierverordnung entspricht und als Klärmittel für Bier eingesetzt werden kann.

Berlin 12.12.1995

VERSUCHS- UND LEHRANSTALT
FÜR BRAUEREI IN BERLIN (VLB)
- Zentral-Laboratorium -

Dr.-Ing. H.-M. Anger

VERSUCHS- UND LEHRANSTALT
FÜR BRAUEREI IN BERLIN

VLB BERLIN

INTERMAG GmbH
Gesellschaft für Brautechnik
Herrn Michael Katzke
Hilgestraße 20

D-55294 Bodenheim

Nachtrag zum Gutachten über die Löslichkeit des Stabilisierungsmittels "Intergarant® Kombi" (Intermag GmbH, Bodenheim)

0 Einleitung

Die geltende Bierverordnung vom 2. Juli 1990 (BGBl, Jg. 1990, Teil 1, S. 1332-1333), zuletzt geändert am 23. November 1993 (BGBl, Jg. 1993, Teil 1, S. 1912), besagt, daß gewerbsmäßig unter der Bezeichnung "Bier" nur Getränke in den Verkehr gebracht werden dürfen, die gegoren sind und den Vorschriften des Vorläufigen Biergesetzes § 9, Abs. 1,2,4 bis 6 und 11 und den §§ 16 bis 19, §20 Abs. 1 Satz 2 und §§21 und 22 Abs.1 der Verordnung zur Durchführung des Vorläufigen Biergesetzes und §4 Abs. 1 u. 2 der neuen Verordnung entsprechen.

Nach diesen Vorschriften (§ 9 Abs. 6) dürfen als Klärmittel nur solche verwendet werden, die mechanisch oder adsorbierend wirken und bis auf gesundheitlich, geruchlich und geschmacklich unbedenkliche, technisch unvermeidbare Anteile wieder ausgeschieden werden.

1 Abgabe von Chloridionen an das Bier nach Stabilisierung mit "Intergarant® Kombi"

Nachdem Zweifel aufgetreten waren, ob das Produkt "Intergarant® Kombi", ein stark anionischer Ionenaustauscher, durch die vorgesehene Art der Regenerierung und erneuten Einsatz ggfs. Chloridionen an das Bier abgibt, wurden zur Klastellung weitere Versuche unternommen.

2 Versuchsaufbau

2.1 Analytik

Die Analytik wurde nach MEBAK Bd. III, 3. Aufl. 1996 Kap 3.10.1 "Chlorid, Sulfat und Phosphat in Bier" ausgeführt.

Verwendung fand ein Hochleistungs-Flüssigkeitschromatograph MERCK-HITACHI: Ionenpaarchromatographie und Leitfähigkeitsdetektor

2.2 Versuchsaufbau

Die Stabilisierungseinrichtung wurde entsprechend Versuchaufbau der Fa. "Intermag" zusammengestellt, mit der Ausnahme, daß die Flüssigkeiten Wasser, Bier, NaCl-Lösung und NaOH-Lösung mittels Treibgas (CO_2) gefördert wurden. Bier wurde aus dem Keg mittels Fitting, die anderen Flüssigkeiten aus einem AfG-Container entnommen.

Die Versuche im einzelnen:

Beladen der Säule mit 20 ml Intergarant® Kombi-Suspension, möglichst luftfrei, entsprechend "Intermag"-Vorschrift [1)]

Unbehandeltes, nicht stabilisiertes Bier, P(robe) 0

Stabilisierungsprogramm:
Leerdrücken der Säule mit 200 ml entionisiertem Wasser

1. Bierlauf 10 Liter Bier, Temperatur 0 - 1 °C, P1

250 ml Wasser
100 ml 2 M NaCl-Lösung
250 ml 1 M NaOH-Lösung
250 ml Wasser
100 ml 1 M NaCl-Lösung
250 ml Wasser

2. Bierlauf 10 Liter Bier, Temperatur 0 - 1 °C, P2

250 ml Wasser
100 ml 2 M NaCl-Lösung
250 ml 1 M NaOH-Lösung
250 ml Wasser
100 ml 1 M NaCl-Lösung
250 ml Wasser
40 ml 1 M NaOH

3. Bierlauf 10 Liter Bier, Temperatur 0 - 1 °C, P3

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1) Intergarant® Kombi. Datenblatt . Fa. Intermag GmbH. Hilgestraße 20. 55294 Bodenheim/Rh.

3 Ergebnisse

Das Verhältnis "Intergarant® Kombi" / Bier entsprach etwa dem Doppelten der für die Praxis vorgesehenen Dosage von ca. 100 ml / hl Bier.

	Chlorid, mg/l
P0:	118
P1:	114
P2:	121
P3:	115

Die Werte liegen unabhängig von der Versuchsanstellung um den P0-Wert im Bereich 118 +/- 4 ppm mit einem Variationskoeffizienten von 2,7%. Dies entspricht den analytischen Fehlerbreiten bei der Hochleistungs-Flüssigkeitschromatographie.

4 Schlußfolgerungen

Im Nachgang zu dem von uns erstellten Gutachten vom 12.12.1995 über die Löslichkeit von Inhaltsstoffen des Stabilisierungsmittels "Intergarant® Kombi" und deren Übergehen in das Bier ist festzustellen, daß bei bestimmungsgemäßer Anwendung keine Chloridionen an das Bier abgegeben werden, selbst wenn "Intergarant® Kombi" in doppelt so hoher Konzentration als in der Praxis erforderlich angewendet wird.

Berlin 27.03.1996

VERSUCHS- UND LEHRANSTALT
FÜR BRAUEREI IN BERLIN (VLB)
- Zentral-Laboratorium -

Dr.-Ing. H.-M. Anger

Expertise about the solubility of the stabilization agent „Intergarant® Kombi“
(Note: “Intergarant Kombi” was the first trade name of Q Sepharose for beer stabilization)

0 Introduction

The valid beer decree from July 2nd, 1990 (BGBl, Y 1990, part 1, p. 1332-1333), last time modified on November 23rd, 1993 (BGBl, Y 1993, part 1, p. 1912), stipulates that marketing a beverage as “Beer” is only permitted if it is fermented and produced in compliance with the preliminary beer law §9, section 1, 2, 4 to 6 and 11 and §§16 to 19, § 20 section 1, sentence 2 and §§ 21 and 22 section 1 of the implementation decree of the preliminary beer law and §4 section 1 and 2 of the new decree.

According to these regulations (§9, section 6) only those finings are permitted which act in a mechanic or adsorbing manner and which can be removed again except technically unavoidable parts which then shall be harmless regarding health, odour and taste.

1 Stabilization agent “Intergarant® Kombi”

The product “Intergarant® Kombi” is a strong anion ion-exchanger which was developed with the trade name “Q Sepharose® Big Beads” by the company Pharmacia Biotech AB, Uppsala, Sweden, mainly for industrial applications in the bio-medical field. The media consists of 100-300 µm particles of high physical stability. The ion-exchange groups are coupled to a highly cross-linked agarose matrix and have a high binding capacity for protein molecules across almost the complete pH-range. It would meet the conditions in beer (pH-range 4,0 to 5,0) where a removal of protein by adsorption contributes to the non-biological shelf life of beer.

The regeneration of the media is done by 2 M sodium chloride solution for ionic bound proteins and by 1 M sodium hydroxide solution for hydrophobe bound proteins or lipoproteins.

Nothing else than the protein-adsorbing features of Q Sepharose in beer respectively under the conditions of beer production were expected, nevertheless it was investigated whether media substances were released by the use of "Intergarant® Kombi" for beer stabilization and by cleaning and regeneration procedures.

For hygienic reasons the product was delivered and stored under a 20% (v/v) ethanol solution.

2 Potential soluble substances of "Intergarant® Kombi"

In the Pharmacia "Regulatory Support File: Q Sepharose Big Beads" [1)], Section 7 substances of the Q Sepharose are listed, which could be extractable under extreme conditions and which originate from the product or are residuals from the manufacturing process.

From these substances were selected:

1 Toluene
2 Epichlorohydrine
3 Allyl glycidyl ether
4 Acetone
5 Ethanol
6 Glucose
7 Hydroxy methyl furfurane

[1)] Regulatory Support File Q Sepharose Big Beads, Vers. No 1.2, 20.02.95, Pharmacia Biotech, Uppsala, Sweden

3 Experimental set-up

3.1 Methods

The methods were partly taken from the “Support File” 1), i.e. the method for the determination of volatile substances (1-5) from section 9 part 5:

A)

Determination of Toluene, Epichlorohydrine, Allyl glycidyl ether, Acetone, Ethanol by headspace gas chromatography:

Gas chromatograph: HEWLETT PACKARD 5890

Column:	30m*0,25.. i.d. capillary column, DB-WAW and FFAP
Oven:	Program 80°C, 0,5 min, heating rate 15°C/min, 200°C, 1 min
Injector:	210°C
Carrier gas:	Hydrogen, approx. 1 ml/min
	Split ratio 1 : 66,
Detector:	220°C
	Flame Ionization Detector (FID)
Operating gas	Hydrogen, 18 psi,
	Synth. Air, 28 psi
Make-up-Gas	Nitrogen, 48 psi
Injection	10 ml Headspace
Integrator	Hewlett Packard

B)

The determination of eventually released glucose was done by a MEBAK method.

Carbohydrates after hydrolysis and enzymatic determination of glucose, hexokinase-method[2)]:

Hydrolysis of gluco-saccharides by 25% HCl (hydrochloric acid)

Enzymatic determination of glucose by Test Combination Glucose, Boehringer, Mannheim.

[2)] Middle European brew technological method committee (MEBAK), H. Pfenniger (Hg.). Freising-Weihenstephan; Selbstverlag der MEBAK, Book II, section 2.38.2, page 246 ff.

C)

Hydroxy methyl furfurane analysis according a method by Wackerbauer et al. 3): Extraction of HMF from beer by ethyl acetate, drying, eluting in solvent.

High Performance Liquid Chromatography

HPLC	Merck-Hitachi
Injector	Rheodyne 7125 (20 µl)
Column	250 mm Nucleosil 5 C_{18}
Elution liquid	Acetonitril : water 15:85 v/v
Flow velocity	1 ml/min^{-1}
Detector	UV 277 nm
Integrator	Merck-Hitachi

3.2 Set-up

The stabilization equipment was assembled according the proposed set-up of the company “Intermag” with the exception that the liquids water, beer, NaCl-solution and NaOH-solution were produced *(tapped)* by gas (CO_2). Beer was taken from a keg by using a fitting, the other liquids were taken from beverage container.

The experiments in detail:
Packing the column according to “Intermag” instructions [4].
Untreated, unstabilised beer, sample P 0

Stabilization program:
Flush column with 250 ml water, P1
100 ml NaCl-solution
250 ml water
250 ml NaOH-solution
250 ml water, P2

First beer processing with 8l/hr for 6 hrs, temperature 0-1°C
Beer sample after 1 hr processing, P3
Beer sample after 2 hr processing, P4
Beer sample after 3 hr processing, P5

[3] Wackerbauer, K., Krämer, P., Methner, F.-J., Marx, U., Mschr. Brauwiss. 36 (11), 439-442, 1983
[4] Intergarant® Kombi, Datenblatt, Fa. Intermag GmbH, Hilgestrasse 20, 55294 Bodenheim/Rh.

Beer sample after 4 hr processing, P6
Beer sample after 5 hr processing, P7
Beer sample after 6 hr processing, P8
250 ml water, P9
500 ml NaCl-solution, reversed flow
250 ml water
1600 ml NaOH-solution (40°C)
500 ml water, P10
Stored the closed and sealed column for 14 days.

Repeat starting *(preparation)* procedure as described above
250 ml water prior beer processing, P11
Second beer processing with 8l/hr for 6 hrs, temperature 0-1°C
Beer sample at start, P12
Beer sample after 1 hr processing, P13
Beer sample after 2 hr processing, P14
Beer sample after 3 hr processing, P15
Beer sample after 4 hr processing, P16
Beer sample after 5 hr processing, P17
Beer sample after 6 hr processing, P18
250 ml water, P19
500 ml NaCl-solution, reversed flow
250 ml water, P20
1600 ml NaOH-solution (40°C), P21
500 ml water, P22

As comparison (to P0 to P22)
beer stabilized with silica gel, P23

Long-time experiments:
Exposure of beer to an Intergarant® Kombi-suspension:
Untreated sample, 7 days at room temperature 22°C, pH 4,51, P24
Beer treated with 60 mg “Intergarant® Kombi”, equal to 12g/hl in 500 ml, 7 days at room temperature 22°C, pH 4,51, P25
Beer treated with 60 mg “Intergarant® Kombi”, equal to 12g/hl in 500 ml, 7 days at room temperature 22°C, pH 4,00 P26
Beer treated with 60 mg “Intergarant® Kombi”, equal to 12g/hl in 500 ml, 7 days at room temperature 22°C, pH 5,00, P27

Exposure of an other beer to an Intergarant® Kombi-suspension:
Untreated sample, 7 days at room temperature 22°C, P28
Beer treated with 60 mg “Intergarant® Kombi”, equal to 12g/hl in 500 ml, 7 days at room temperature 22°C, P29

4 Results

Beer samples (Tap beer, Pilsener type)
Original gravity: 11,04% w/w
Apparent extract: 2,64% w/w
Real extract: 4,24% w/w
Alcohol: 3,49% w/w (=4,45% v/v)
Fermentation rate: 76,9%
pH-value: 4,51

Sample	Acetone ppm	Toluene[5)] ppm	Epichlorhydrin[5)] ppm	Allyl glycidyl ether ppm	Ethanol[6)] ppm	Glucose[7)] g/100ml	HMF ppm
P0	n.d	n.d.	n.d.	n.d.	n.d.	2,72	1,7
P1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P3	n.d.	n.d.	n.d.	n.d.	n.d.	2,81	1,7
P4	n.d.	n.d.	n.d.	n.d.	n.d.	3,24	1,8
P5	n.d.	n.d.	n.d.	n.d.	n.d.	2,68	1,8
P6	n.d.	n.d.	n.d.	n.d.	n.d.	3,15	1,7
P7	n.d.	n.d.	n.d.	n.d.	n.d.	2,94	1,4
P8	n.d.	n.d.	n.d.	n.d.	n.d.	2,98	1,3
P9	n.d.	n.d.	n.d.	n.d.	n.d.	0,05	n.d.
P10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P12	n.d.	n.d.	n.d.	n.d.	n.d.	2,81	1,3
P13	n.d.	n.d.	n.d.	n.d.	n.d.	2,89	1,3
P14	n.d.	n.d.	n.d.	n.d.	n.d.	2,68	1,5
P15	n.d.	n.d.	n.d.	n.d.	n.d.	2,76	1,3
P16	n.d.	n.d.	n.d.	n.d.	n.d.	2,81	1,4
P17	n.d.	n.d.	n.d.	n.d.	n.d.	2,94	1,3
P18	n.d.	n.d.	n.d.	n.d.	n.d.	3,11	1,6
P19	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. not detected under the experimental conditions.

[5)] Toluene, Epichlorohydrine: overlapping peaks with other, not identified beer compounds on DB-WAX column, separation on column with different polarity.
[6)] No significant increase of beer ethanol from 4,45% v/v (=44 500 ppm + x) detected.
[7)] Total glucose after hydrolysis of all starch rests (Dextrine)

Sample	Acetone ppm	Toluene[5)] ppm	Epichlorhydrin[5)] ppm	Allyl glycidyl ether ppm	Ethanol[6)] ppm	Glucose[7)] g/100ml	HMF ppm
P23	n.d	n.d.	n.d.	n.d.	n.d.	2,89	1,5
P24	n.d.	n.d.	n.d.	n.d.	n.d.	2,90	2,0
P25	n.d.	n.d.	n.d.	n.d.	n.d.	2,91	1,9
P26	n.d.	n.d.	n.d.	n.d.	n.d.	2,92	1,9
P27	n.d.	n.d.	n.d.	n.d.	n.d.	2,89	1,9
P28	n.d.	n.d.	n.d.	n.d.	n.d.	3,33	5,5
P29	n.d.	n.d.	n.d.	n.d.	n.d.	3,63	5,9

n.d. not detected under the experimental conditions.

5 Conclusion

The product “Intergarant® Kombi” from Intermag GmbH, Bodenheim, used under usual conditions of beer stabilization (beer, pH, temperature) as well as under extreme conditions (pH, temperature, contact time) showed after the investigation of the beer samples the following results

- no detection respectively no increase of volatile substances;
- with variations (analytical error / inaccuracy) in the range of 0,2-0,3% no significant correlation between the determined glucose content and the beer stabilization by Intergarant® Kombi,
- Considering the analytical error *(inaccuracy)* no impact on the HMF content caused by the beer stabilization.

Generally, a solubility of the fining in beer could not be established. Therefore it is concluded that the product Intergarant® Kombi complies with above mentioned Beer Decree and is permitted as beer fining.

Berlin 12.12.1995

VLB …

Supplement to the expertise about the solubility of the stabilization agent „Intergarant® Kombi“ (Intermag GmbH, Bodenheim)

0 Introduction

The valid beer decree from July 2nd, 1990 (BGBl, Y 1990, part 1, p. 1332-1333), last time modified on November 23rd, 1993 (BGBl, Y 1993, part 1, p. 1912), stipulates that marketing a beverage as “Beer” is only permitted if it is fermented and produced in compliance with the preliminary beer law §9, section 1, 2, 4 to 6 and 11 and §§16 to 19, § 20 section 1, sentence 2 and §§ 21 and 22 section 1 of the implementation decree of the preliminary beer law and §4 section 1 and 2 of the new decree.

According to these regulations (§9, section 6) only those finings are permitted which act in a mechanic or adsorbing manner and which can be removed again except technically unavoidable parts which then shall be harmless regarding health, odour and taste.

1 Release of chloride ions into the beer after stabilization with „Intergarant® Kombi“

Since concerns came up whether the product „Intergarant® Kombi“, which is a strong anion ion-exchanger, could eventually release chloride ions into the beer due to the method of regeneration and repeated use, more experiments were done.

2 Set-up of experiments

2.1 Methods

The analysis was done according MEBAK book III, 3. edition 1996, section 3.10.1 “chloride, sulfate and phosphate in beer”.
A High Performance Liquid Chromatrograh MERCK-HITACHI was used: Ion-pair chromatography and conductivity detection.

2.2 Set-up of experiments

The stabilization equipment was assembled according the proposed set-up of the company “Intermag” with the exception that the liquids water, beer, NaCl-solution and NaOH-solution were produced *(tapped)* by gas (CO_2). Beer was taken from a keg by a fitting, the other liquids were taken from beverage container.

The experiments in detail:
Packing the column with 20 ml Intergarant® Kombi-solution according to “Intermag” instructions [1)].

Untreated, unstabilised beer, sample P 0

Stabilization program:
Flush column with 200 ml de-mineralized water

First processing with 10 liter beer, temperature 0-1°C, P1
250 ml water
100 ml 2 M NaCl-solution
250 ml 1 M NaOH-solution
250 ml water
100 ml 1 M NaCl-solution
250 ml water

Second processing with 10 liter beer, temperature 0-1°C, P2
250 ml water
100 ml 2 M NaCl-solution
250 ml 1 M NaOH-solution
250 ml water
100 ml 1 M NaCl-solution
250 ml water
40 ml 1 M NaOH

Second processing with 10 liter beer, temperature 0-1°C, P3

[1)] Intergarant® Kombi data sheet, Intermag GmbH, Hilgestrasse 20, 55294 Bodenheim/Rh.

3 Results

The ratio “Intergarant® Kombi” / beer was approximately twice as much as the usual practical dosage of approximately 100 ml / hl beer.

	Chloride, mg/l
P0:	118
P1:	114
P2:	121
P3:	115

The values are independent from the experimental set-up on the P0-value level and in a range of 118 +/- 4 ppm with a statistical variation of 2,7%. That is within the range of analytical error typical for High Performance Liquid Chromatography.

4 Conclusion

Supplementary to our expertise from 12.12.1995 about the solubility of ingredients of the stabilization agent “Intergarant® Kombi” and it’s release into the beer it is stated that by normal usage no chloride ions are released into the beer, even if Intergarant® Kombi is used in a concentration which is twice as high as needed in practice.

Berlin 27.03.1996

VLB……..

Министерство здравоохранения
Российской Федерации
Наименование учреждения
Сев.-Зап. рег.ЦГСЭН на трансп.

Код формы по ОКУД
Код учреждения по ОКПО
Медицинская документация
Форма № 303-00-3/у
Утверждено приказом
Министерства здравоохранения
Российской Федерации
от 27.10.2000 № 381

ГОСУДАРСТВЕННАЯ САНИТАРНО-ЭПИДЕМИОЛОГИЧЕСКАЯ СЛУЖБА РОССИЙСКОЙ ФЕДЕРАЦИИ
ГЛАВНЫЙ ГОСУДАРСТВЕННЫЙ САНИТАРНЫЙ ВРАЧ
ПО СЕВЕРО-ЗАПАДНОМУ РЕГИОНУ НА ТРАНСПОРТЕ
(наименование территории, ведомства)

САНИТАРНО-ЭПИДЕМИОЛОГИЧЕСКОЕ ЗАКЛЮЧЕНИЕ

№ 78.02.03.513.П.003398.10.03 от 21.10.2003 г.

Настоящим санитарно-эпидемиологическим заключением удостоверяется, что производство, применение (использование) и реализация новых видов продукции; продукция, ввозимая на территорию Российской Федерации
АДСОРБЕНТ ДЛЯ ФИЛЬТРОВАНИЯ НАПИТКОВ, тип: CSS

изготовленная в соответствии
Декларация соответствия изготовителя от 05.06.2001г. Таблица безопасности.

СООТВЕТСТВУЕТ ~~(НЕ СООТВЕТСТВУЕТ)~~ государственным санитарно-эпидемиологическим правилам и нормативам (ненужное зачеркнуть, указать полное наименование санитарных правил)
ГН 2.3.3.972-00 "Предельно допустимые количества химических веществ, выделяющихся из материалов, контактирующих с пищевыми продуктами".

Организация — изготовитель
"ALBERT HANDTMANN, ARMATURENFABRIK GMBH & CO KG", Arthur-Handtmann-Str, 13+23, D-88400 Biberach ФРГ
Получатель санитарно-эпидемиологического заключения
"ALBERT HANDTMANN, ARMATURENFABRIK GMBH & CO KG", Arthur-Handtmann-Str, 13+23, D-88400 Biberach ФРГ
Основанием для признания продукции, соответствующей ~~(не соответствующей)~~ государственным санитарно-эпидемиологическим правилам и нормативам являются (перечислить рассмотренные протоколы исследований, наименование учреждения, проводившего исследования, другие рассмотренные документы):
Протоколы испытаний ИЛЦ ЦГСЭН на транспорте (водном и воздушном) в Северо-Западном регионе № ОСН/КПП/716 от 17.10.2003г.

№ 0810990

Гигиеническая характеристика продукции

Вещества, показатели (факторы)	Гигиенический норматив (СанПиН, МДУ, ПДК и т.д.)
Материал: натуральные карбогидраты морских водорослей, этанол	
Формальдегид	0,1 мг/л
Бензол	0,01 мг/л
Этилацетат	0,2 мг/л
Этиловый спирт	0,2 мг/л
Свинец	0,03 мг/л
Ртуть	1,0 мг/л
Кадмий	1,0 мг/л

Область применения:

Адсорбент для использования в пивоваренной промышленности.

Необходимые условия использования, хранения, транспортировки и меры безопасности:

В соответствии с инструкцией по применению .

Информация, наносимая на этикетку:

Название, страна, фирма-изготовитель, назначение, инструкция по применению на русском языке.

Заключение действительно до 20.10.2008 г.

Главный государственный санитарный врач
(заместитель главного государственного санитарного врача)

Ф.И.О. Куракова Л.В.

Бланк N 0810990

Формат А4. Бланк. Срок хранения 5 лет.

STATE SANITARY-EPIDEMIOLOGICAL AGENCY OF RUSSIA

Ministry of Healthcare of the
Russian Federation

Form code by ОКУД
Form code by ОКПО
Medical documents
Form # 306-06-5/y
Approved by the Ministry of Healthcare
of the Russian Federation
of 27 October 2006 # 581

Name of the Institution
North-West Regional CSSEI on Transport

STATE SANITARY-EPIDEMIOLOGICAL AGENCY OF THE RUSSIAN FEDERATION
CHIEF STATE SANITARY PHYSICIAN
IN NORTH-WEST REGION ON TRANSPORT
Name of the Institution, Department

SANITARY-EPIDEMIOLOGICAL CERTIFICATE

78.02.03.513.П 003396.10.03 of 21 October 2003

This Sanitary-Epidemiological Certificate certifies that manufacture, application (use) and selling of new types of produce, products imported into the Russian Federation territory

ADSORBENT FOR FILTERING DRINKS, type CSS

MANUFACTURED IN COMPLIANCE WITH

The Manufacturer's Declaration of Compliance of 05 June 2001. Safety Table.

COMPLIES (~~DOES NOT COMPLY~~) with the State sanitary-epidemiological rules and standards (cross out the unnecessary, indicate full name of the sanitary rules).

ГН 2.3.3.972-00 "Prohibitive amounts of chemical substances released from the materials contacting with food".

Organisation – manufacturer

"ALBERT HANDTMANN, ARMATURENFABRIK GMBH & CO KG", Arthur-Handtmann-Str, 13+23, D-88400 Biberach FRG

Receiver of the SANITARY-EPIDEMIOLOGICAL CERTIFICATE

"ALBERT HANDTMANN, ARMATURENFABRIK GMBH & CO KG", Arthur-Handtmann-Str, 13+23, D-88400 Biberach FRG

The grounds for recognition of the produce as complying (~~not complying~~) with the State sanitary-epidemiological rules and standards involve (indicate the considered protocols of studies, name of the institution that performed the studies, other documents under consideration):

Protocols of trials conducted by the Trial-Laboratory Centre of the CSSEI on Transport (water transport and air transport) in the North-West Region #ОСНЛКПП/716 of 17 October 2003.

 # 0810990

Hygienic characteristics of the produce

Substances, parameters (factors)	Hygienic standard (Sanitary Rules and Standards, Maximum allowed level, Maximum allowed concentration, etc.
Material: natural seaweed carbohydrates, ethanol	
Formaldehyde	0.1 mg/L
Benzene	0.01 mg/L
Ethyl acetate	0.2 mg/L
Ethyl alcohol	0.2 mg/L
Lead	0.03 mg/L
Mercury	1.0 mg/L
Cadmium	1.0 mg/L

Field of application:
Adsorbent for using in brewery.

The necessary conditions of use, storage, shipping, and safety measures:
According to the instruction manual for use

The information placed on the label:
Name, country, company-manufacturer, designation, instruction manual in Russian language.

The Certificate is valid till
20 October 2008 ***LABEL***

Chief State Sanitary Physician (Signed)
(Deputy Chief State Sanitary Physician)

Form # 0810990

SEAL: **Ministry of Healthcare of the Russian Federation**
North-West Regional CSSEI on Transport

MINISTRY OF HEALTHCARE OF THE RUSSIAN FEDERATION
STATE INSTITUTION "THE CENTRE OF SANITARY-EPIDEMIOLOGICAL INSPECTION ON TRANSPORT (WATER AND AIR TRANSPORT) IN NORTH-WEST REGION"

The Trial-Laboratory Centre

Juridical address: 198035, St. Petersburg, 6 Gapsalskaya St.
Telephone: (812) 251-07-98, Fax: (812) 251-72-98

Accreditation Attestation:
№ ГСЭН.RU.ЦОА.III of 13 August 2002
Registered in the RF State Register
№ РОСС.RU.0001.510244

"I APPROVE"

Head of the TLC
(Signed) I.V. Parfenova
17 October 2003

SEAL: **Ministry of Healthcare of the Russian Federation**
State Institution "The Centre of State Sanitary-
Epidemiological Inspection on Transport
(water and air transport) in North-West Region

P R O T O C O L

OF LABORATORY STUDIES

 # ОСН | КПП | 716 of **17 October 2003**

1. Name of the organization-applicant **Closed joint-stock company "SZhS Vostok Ltd.**
2. Name of the sample (assay) **Adsorbent type CSS**
3. Manufacturer (company, enterprise, organization) **"Albert Handtmann, Armaturenfabrik GmbH & Co.Kg" Germany**
5. Delivered to the TLC **09 October 2003**
6. Name, position **Physician Korotkevich, A.V.** Delivery conditions **auto truck transport**
7. Additional information **Material: natural seaweed carbohydrates, ethanol**
8. Normative documents regulating volume of the laboratory studies and their evaluation **ГН 2.3.3.972-00**

Parameters under study	Measurement units	Results of the studies		Standard parameter values	Normative documents for study methods
		Absolute values	Error		
Chemical studies	Registration number in the journal **520/356**				
The extract characteristics					
- odour		0		2	GOST 3351-74
- oxidability	mgO_2/L	0.16	± 50%	5.0	ЦВ 1.01.14-98 "А"
Chemical substances					
- formaldehyde	mg/L	less than 0.01	--	0.1	МУК 4.1.653-96
- benzene	mg/L	less than 0.002	--	0.01	МУК 4.1.650-96
- acetone	mg/L	less than 0.01	--		МУК 4.1.650-96
- ethyl acetate	mg/L	less than 0.01	--	0.2	МУК 4.1.650-96
- ethyl alcohol	mg/L	0.4	± 0.1	0.2	МУК 4.1.650-96
- lead	mg/L	0.00981	± 0.00322	0.03	ПНДФ 14.1.2.22-95
- mercury	mg/L	0.0001	± 0.0001	1.0	ПНДФ 14.1.2.22-95
- cadmium	mg/L	0.00037	± 0.00061	1.0	ПНДФ 14.1.2.22-95

Note: a model medium was used in the study: distilled water, 2% solution of citric acid

Official responsible for the Protocol composition (Signed) A.A.Shcherbakova

CONCLUSION

The sample COMPLIES with the requirements ГН 2.3.3.972-00

Head of the Hygienic Expertise Department (Signed) A.V.Korotkevich