UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Lonza Inc.
17-17 Route 208
Fair Lawn, NJ 07410

AUG 23 2000

Subject: Lonza Formulation R-82
EPA Registration No. 6836-78
Amendment Date: 2/23/00
EPA Receive Date: 2/25/00

Attention: Ms. Ruth Trager

The amendment, referenced above, submitted in connection with registration under FIFRA section 3(c)(7)(A) to add an additional organism, Hepatitis B Virus, (HBV), to the label is unacceptable for the following reasons.

The submitted efficacy data, MRID No.450543-01, is not acceptable because the study did not include validation by an independent laboratory, and a positive control was not tested.

The Agency will shortly publish guidance on HBV testing guidelines. We suggest that you review this guidance before doing any further testing to support a HBV claim. If you have any questions regarding this letter, please contact Jacqueline Campbell at (703) 308-6416.

Sincerely,

Velma Noble

Velma Noble
Product Manager (31)
Regulatory Management Branch I
Antimicrobials Division (7510C)

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CONCURRENCES

SYMBOL	7510C							
SURNAME	Campbell							
DATE	8/23/00							

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

August 23, 2000

MEMORANDUM

Subject: Efficacy Review for Lonza Formulation R-82 (EPA Reg. No. 6836-78)
DP Barcode: D 264023

From: Ibrahim S. Barsoum, Ph.D., Microbiologist
Product Science Branch
Efficacy Evaluation Team
Antimicrobials Division (7510C)

To: Velma Noble PM31/Jacqueline Campbell
Regulatory Management Branch I
Antimicrobials Division (7510C)

Thru: Emily Mitchell, M.S., Team Leader
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510C)

Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510C)

Applicant: Lonza Inc.
Fair Lawn, NJ, 07410

Formulation From Label:

Active Ingredient(s)	% by wt.
Octyl decyl dimethyl ammonium chloride	6.51%
Dioctyl dimethyl ammonium chloride	2.604%
Didecyl dimethyl ammonium chloride	3.906%
Alkyl dimethyl benzyl ammonium chloride	8.680%
Inert Ingredient(s)	78.300%
Total	100.000%

I. BACKGROUND:

Product Manager has requested to review efficacy data (MRID # 450543-01) to add an additional organism, Hepatitis B virus (HBV) to the label claim. The data which is intended to demonstrate virucidal effectiveness of Lonza Formulation R-82, EPA Registration No. 6836-78, against human HBV was developed using a surrogate test virus, Duck Hepatitis B Virus (DHBV) and an *in-vivo* system to measure disinfectant efficacy against infectious DHBV particles.

II Use Directions

Lonza Formulation R-82 is a hospital disinfectant and virucide when used at 1:256 dilution (½ oz per gallon) in the presence of 400 ppm hard water and 5% organic serum. The product is also used as a sanitizer for hard nonporous non-food contact surfaces in the presence of 500 ppm hard water and 5% organic serum. It is also used as a mildewstat at 1:256 dilution. The registrant is amending the label claims to include effectiveness against HBV at 1:256 dilution and has provided efficacy data results using an *in-vivo* Duck HBV protocol.

III Agency Standards for Proposed Claims

For virucides whose use-directions identify the product as one intended for use upon dry, inanimate, environmental surfaces (such as floors, tables, medical equipment surfaces, etc.), carrier methods, which are modifications of either the AOAC Use-Dilution Method (for liquid surface disinfectants) or the AOAC Germicidal Spray Products Test (for surface spray disinfectants), must be used in the development of the virological data. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of two different batches of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface (petri dish, glass slide, steel cylinder, etc.) for a specified exposure period at room temperature. The virus is then assayed by an appropriate virological technique.

In the case of HHBV there are no *in vitro* systems available for growing the virus. Because the only suitable *in vivo* host for growing HHBV is either humans or chimpanzees, it is not practical to conduct adequate testing of antimicrobial products by directly using HHBV. Therefore, the Agency is exploring the use of appropriate surrogate viruses for use in testing the effectiveness of antimicrobial products against HHBV.

IV Summary of the Submitted Efficacy Studies

MRID # 450543-01

The purpose of this efficacy study was to evaluate the virucidal effect of Lonza Formulation R-82 against HBV using an *in-vivo* Duck HBV (DHBV) protocol. A film of virus

v B

dried on a glass surface was exposed to the disinfectant for a 10 min contact time. After the exposure time, the virus-disinfectant mixtures were detoxified, diluted, and titrated in young ducklings (2 days of age) which have been demonstrated to be free of DHBV using 4 ducklings for each dilution. A total of 126 ducklings were used in the assay. The resuspended virus control film and each batch of disinfectant alone were treated in the same manner. Ducklings were observed for 2.5 weeks with serum samples taken one day pre-inoculation, 1, 1.5, 2.0, 2.5 weeks post inoculation, and liver samples collected at sacrifice from select ducklings. Serum samples were evaluated for the presence of DHBV DNA by blot hybridization. Viral titer and disinfectant cytotoxicity controls were also run concurrently.

Results of this study show that both batches of Lonza Formulation R-82 (5600-18 and 5600-21) reduced the titer of infectious duck HBV by over 4.2 Log_{10} at 1:256 dilution in the presence of 400 ppm hard water and 10% organic soil load in 10 minutes contact time using the DHBV *in-vivo* assay protocol.

V. Conclusions and Recommendations.

There is a broad consensus by the scientific community that duck HBV (DHBV) is an acceptable surrogate for human HBV (HHBV). The scientific evidence indicates that the HHBV and DHBV are closely related. They share considerable structural similarities including a lipid envelop which is highly sensitive to inactivation by a wide variety of disinfectants. Consequently, they have been classified together in the same virus taxonomic family, *Hepadnaviridae*. Chemical disinfectants being considered for this virucidal use have multiple inhibitory effects, such as; protein denaturation, protein cross linking, lipid removal, and nucleic acid degradation. Any one of these activities is sufficient to independently inactivate enveloped viruses. Also, modern disinfectants are typically used at concentrations which are many times greater than that required to kill enveloped viruses. Thus, the conservative expectation is that different Hepadnaviruses will exhibit comparable sensitivity to a given disinfectant. This is particularly true for human and duck HBVs. The Agency is adopting, where possible, policies and data requirements that minimize animal testing, and when animal testing must be conducted, EPA is committed to reducing the number of animals needed for testing, reducing the pain and suffering of the test animals, and whenever scientifically-defensible, replacing animals with validated non-animal test systems.

The submitted data was generated using a protocol that lacked sufficient controls to demonstrate the statistical adequacy of the data. In addition, the Agency is requiring new protocols to undergo validation as a component of the registration process. Due to these deficiencies, the Applicant is required to submit additional efficacy data according the guidance provided below. In keeping with the Agency's commitment to reduce or eliminate the number of animals needed for testing, it is recommended that all additional testing be conducted using an *in vitro* assay system.

1. Data must be generated using duplicate positive control carriers and duplicate test carriers for each of the two product batches.

2. The data to be generated would also require the use of a common positive control disinfectant to be tested concurrently with the product. This agent should serve as both an intra- and an inter-laboratory control and will be used for analyzing reproducibility of the efficacy data.

3. The product needs to be retested using 2 lots of Lonza Formulation R-82 and the positive control disinfectant BTC-835.

4. In addition, confirmatory testing using one of the product lots along with the BTC control must be done in a second testing facility.

5. The Agency is finalizing a Federal Register Notice which will provide additional guidance to the regulated community on acceptance of HBV data using surrogate duck assays.