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ORIGINAL SUBMISSION

000304

26 July, 2000



Linda S. Kahl, Ph.D.
Regulatory Policy Branch, HFS-206
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W.
Washington, D.C. 20204

Dear Dr. Kahl,

We are hereby submitting, in triplicate, a generally recognized as safe (GRAS) notification, in accordance with proposed 21 C.F.R. § 170.36, for Novo Nordisk's xylanase enzyme preparation produced by a selected strain of *Fusarium venenatum* expressing the gene encoding a xylanase (endo 1,4- β -xylanase) from *Thermomyces lanuginosus* for use as a processing aid in the baking industry. This includes a GRAS Notification exemption claim and a summary of information supporting the GRAS determination. Please find enclosed three binders, containing one original and two copies of this information.

Please contact me by direct telephone at 919 494-3152, direct fax at 919 494-3420, or email at shs@novo.dk if you have any questions or require additional information.

Sincerely,

Scott H. Shore, Ph.D.
Senior Regulatory Specialist

Enclosures (3 binders)

2000 JUL 28 P 1: 16

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Office of Premarket Approval
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W.
Washington, D.C. 20204

Novo Nordisk



**Novo Nordisk BioChem
North America, Inc.**

77 Perry Chapel Church Road
Box 576
Franklinton, NC 27525-0576

Tel. 919-494-3000
FAX 919-494-3450

RE: GRAS Notification - Exemption Claim

Pursuant to the proposed 21C.F.R. §170.36(c)(1) Novo Nordisk BioChem North America Inc. hereby claims that a xylanase enzyme preparation produced by submerged fermentation of a selected strain of *Fusarium venenatum* carrying the gene encoding a xylanase from *Thermomyces lanuginosus* is Generally Recognized as Safe; therefore, it is exempt from statutory premarket approval requirements.

The following information is provided in accordance with the proposed regulation:

Proposed §170.36(c)(1)(i) *The name and address of the notifier.*

Novo Nordisk BioChem North America Inc.
77 Perry Chapel Church Rd., Box 576
Franklinton, NC 27525

Proposed §170.36(c)(1)(ii) *The common or usual name of notified substance.*

Xylanase enzyme preparation from a selected non-toxicogenic strain of *Fusarium venenatum* carrying the gene encoding xylanase from *Thermomyces lanuginosus*.

Proposed §170.36(c)(1)(iii) *Applicable conditions of use.*

The above described xylanase preparation is to be used as a processing aid for baking. The enzyme preparation is used at minimum levels necessary to achieve the desired effect and according to requirements for normal production following Good Manufacturing Practices.

Proposed §170.36(c)(1)(iv) *Basis for GRAS determination.*

This GRAS determination is based on scientific procedures.

Proposed §170.36(c)(1)(v) *Availability of information.*

A notification package providing a summary of the information which supports this GRAS determination is enclosed with this letter. The package includes a safety evaluation of the production strain, the enzyme, and the manufacturing process, as well as an evaluation of dietary exposure. Complete data and information that are the basis for this GRAS determination are available to the Food and Drug Administration for review and copying upon request at the address above or copies of the material can be sent to FDA upon request.

John Carroll
Director, Regulatory Affairs

Date

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NOVOZYM[®] 899

**A xylanase enzyme preparation produced by
a selected strain of *Fusarium venenatum* expressing
the gene encoding a xylanase from *Thermomyces lanuginosus***

Scott H. Shore
Enzyme Regulatory Affairs
Novo Nordisk BioChem N.A., Inc.

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TABLE OF CONTENTS

	<u>PAGE</u>
1. EXPERT PANEL REPORT	3
2. INTRODUCTION	4
3. PRODUCTION MICROORGANISM	6
3.1 Production Strain	6
3.2 Recipient Microorganism	6
3.3 Introduced DNA Sequences	7
3.4 Construction of the Production Strain LyMC4.B	12
3.5 Antibiotic Resistance Gene	14
3.6 Stability and Transfer Capability of the Introduced DNA	15
3.7 Plant Pathogenicity	15
3.8 Human Pathogenicity	15
3.9 Mycotoxins	15
4. ENZYME IDENTITY and CHARACTERIZATION	18
4.1 Enzyme Identity	18
4.2 Enzymatic Activity	18
5. MANUFACTURING PROCESS	19
5.1 Raw Materials	19
5.2 Fermentation	19
5.3 Recovery	23
6. COMPOSITION and SPECIFICATIONS	25
6.1 Composition	25
6.2 Specifications	25
6.3 Absence of the Production Strain	25
7. APPLICATION	26
7.1 Technological Function	26
7.2 Use Levels	26
7.3 Enzyme Residues in the Final Food	26
8. SAFETY EVALUATION	27
8.1 Production Strain	27
8.2 Enzyme Component	27
8.3 Manufacturing Process	28
8.4 Safety Studies	29
8.5 Estimates of Human Consumption and Safety Margin	30
8.6 Conclusion	31
9. LIST OF APPENDICES	32
APPENDIX A	General References
APPENDIX B	Production Strain References
APPENDIX C	Enzyme Identity and Characterization References
APPENDIX D	Manufacturing Process References
APPENDIX E	Safety Reference



Novo Nordisk

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1. EXPERT PANEL REPORT

Novo Nordisk requested an independent panel of recognized experts (Expert Panel), qualified by their scientific and/or medical training and relevant national and international experience to evaluate the safety of food and food ingredients to determine the generally recognized as safe (GRAS) status of a xylanase enzyme preparation produced by a selected strain of *Fusarium venenatum* expressing the gene encoding a xylanase (endo 1,4- β -xylanase) from *Thermomyces lanuginosus* for use as a processing aid in baking.

The Expert Panel unanimously concluded that, under specified conditions of use, the described xylanase preparation, meeting appropriate food grade specifications and produced in accordance with current good manufacturing practices, is GRAS based on scientific procedures. The Expert Panel reached this conclusion based on the expert's knowledge, experience, and independent and critical evaluation of both published and unpublished reference materials, including internal Novo Nordisk data, documentation, and reports. The Expert Panel's report is included as Attachment 1-A.

The members of the Expert Panel are:

Dr. Joe Borzelleca, Chair
Department of Pharmacology and Toxicology
Medical College of Virginia
Virginia Commonwealth University
Richmond, VA

Dr. Bruce Jarvis
Department of Chemistry and Biochemistry
University of Maryland
College Park, MD

Dr. Mike Pariza
Food Research Institute
Department of Food Microbiology and Toxicology
University of Wisconsin
Madison, WI

Dr. Tim Phillips
Veterinary Anatomy and Public Health
College of Veterinary Medicine
Texas A&M University
College Station, TX

Dr. Bob Proctor
USDA ARS NCAUR
Peoria, IL

000311

EXPERT PANEL REPORT ON THE GENERALLY RECOGNIZED AS SAFE (GRAS) USE OF A XYLANASE ENZYME PREPARATION PRODUCED BY A NON-TOXIGENIC *FUSARIUM VENENATUM* STRAIN EXPRESSING THE GENE ENCODING A XYLANASE FROM *THERMOMYCES LANUGINOSUS*

27 January 2000

The undersigned, an independent panel of recognized experts (hereinafter the Expert Panel), qualified by their scientific and/or medical training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by Novo Nordisk to determine the generally recognized as safe (GRAS) status of a xylanase enzyme preparation produced by *Fusarium venenatum* expressing the gene encoding a xylanase (endo 1,4- β -xylanase) from *Thermomyces lanuginosus* for use as a processing aid in baking (hereinafter the enzyme preparation). The qualifications of the members of the Expert Panel are evidenced in their *curricula vitae*, which appear in Reference List A.

Novo Nordisk compiled a documentation package supporting the safety of the enzyme preparation and submitted this information to the panel in December 1999. This documentation includes scientific evidence from selected journal articles and other publications as well as internal Novo Nordisk documentation. In addition, the statements and conclusions are based on knowledge of the published literature, up to December 1999, on xylanases, *Fusarium* species, mycotoxins, enzymes used in food, and guidelines for assessing the safety of food ingredients from genetically modified microorganisms.

Following independent and critical evaluation of these materials, the Expert Panel conferred several times and then met in Chicago, IL on 27 January 2000, with representatives from Novo Nordisk. The Expert Panel then met in executive session and unanimously concluded that, under specified conditions of use, the described xylanase preparation, meeting appropriate food grade specifications and produced in accordance with current good manufacturing practices, is GRAS based on scientific procedures. This report summarizes data and information critically evaluated by the Expert Panel and that formed the basis for the conclusion of the Expert Panel.

1. Introduction

The xylanase enzyme preparation is produced by submerged fermentation of *Fusarium venenatum* expressing the gene encoding a xylanase from *Thermomyces lanuginosus*.

This xylanase enzyme preparation is to be used in the food industry as a processing aid for baking applications.

The enzyme is an endo-1,4- β -xylanase. It hydrolyzes xylosidic linkages in an arabinoxylan backbone resulting in depolymerization of the arabinoxylan into smaller oligosaccharides. This increases the elasticity of the gluten network, improving handling and stability of the dough.

The following sections summarize the documentation evaluated by the Expert Panel. This includes information on the production microorganism, the enzyme identity and characteristics, the manufacturing process, the product composition and specifications, the proposed usage of the enzyme preparation, the biological studies and the safety evaluation performed. Finally, the summary of the evaluation and the conclusion drawn by the Expert Panel is presented.

2. Production Microorganism

The production strain is derived from a well-known culture collection strain (ATCC 20334), which has been cultured as a mycoprotein source for human consumption in England for more than 20 years under the registered trade name Quorn (Trinci, 1992, Reference C-1). It was originally deposited as a strain of *Fusarium graminearum*. However, thorough investigations of the taxonomy by O'Donnell et al. (1998, Reference C-2) and Yoder and Christianson (1998, Reference C-3) have revealed, that the strain belongs to the species *Fusarium venenatum* established by Nirenberg (1995, Reference C-4).

The genetically modified *F. venenatum* production strain meets the criteria for a safe production microorganism initially outlined by Pariza and Foster (1983, Reference B-1) and further developed by several expert groups (References B 2-8: Berkowitz and Maryanski, 1989; International Food Biotechnology Council, 1990; EU Scientific Committee for Food, 1991; Organisation for Economic Cooperation and Development, 1992 and 1993; FAO/WHO, 1996; ILSI Europe Novel Food Task Force, 1996). These criteria include the identification and characterization of the host strain, plasmid vectors, and inserted genetic sequences.

The *F. venenatum* production strain was constructed by common transformation procedures using well-known plasmid vectors with strictly defined and well-characterized DNA sequences that are not known to encode or express any harmful or toxic substances. Two plasmids were used in the strain construction, one a xylanase expression plasmid and the other a *trf5* deletion plasmid. The development of the production strain was evaluated at every step to assess incorporation of the desired functional genetic information and to ensure no unintended sequences were incorporated.

The selectable markers incorporated to facilitate strain selection were assessed according to the criteria described by several expert groups (References B 2-8). This includes a determination that they do not add toxic components to the food supply and because the production microorganism is not contained in the commercial enzyme preparation and the production microorganism is not released to the environment there is no potential for gene transfer to other organisms.

The xylanase expression plasmid is composed of the endo-1,4- β -xylanase gene from *T. lanuginosus* fused to the *Fusarium oxysporum* trypsin gene promoter and terminator (Royer, et al. 1995, Reference C-5), the *Escherichia coli* plasmid vector pUC19 (Yanish-Perron et al.

1985, Reference C-6), and a 1.8 kb fragment consisting of the *Streptomyces hygrosopicus* bar gene (Thompson, et al. 1987, Reference C-7) fused to the *Aspergillus nidulans* amdS promoter (Corrick, et al. 1987, Reference C-8) and the *Aspergillus niger* glucoamylase terminator (Boel, et al. 1984, Reference C-9).

A tri5 deletion plasmid was designed to incorporate a deleted replacement of the trichodiene synthetase gene, rendering it incapable of producing secondary metabolites within the trichothecene pathway (Royer et al. 1999, Reference C-10).

This strain of *F. venenatum*, is regarded as non-pathogenic and non-toxigenic. In addition to the gene deletion of the trichodiene synthetase, extensive investigations of the metabolic potential indicate that the production strain does not produce secondary metabolites of toxicological concern to humans or higher animals (Miller and MacKenzie; in press, Reference C-11 and documentation provided by Novo Nordisk).

F. venenatum occurs on *Humulus lupulus*, *Solanum tuberosum*, *Spinacia oleracea*, *Triticum aestivum*, *Zea mays* and in soil. Strain ATCC 20334 (= A 3/5) was found by Novo Nordisk to be non-pathogenic on potato, barley and wheat. *F. venenatum* is not considered a human pathogen.

The strain has reportedly a long history of safe industrial use. Biomass of *F. venenatum* ATCC 20334 (=A3/5) is marketed as Quorn Mycoprotein and has been a readily available human food source in England since 1985 (Reference C-1). The Quorn Mycoprotein was concluded to be a safe food ingredient in an expert panel report, that has been used to support a Food Additive Petition in USA (personal communication from Marlow Foods to Novo Nordisk). The Novo Nordisk production strain derivative has been modified to further reduce its toxicological potential.

3. Enzyme Identity and Characterization

The primary enzyme activity is endo-1,4- β -xylanase. According to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUB), "Enzyme Nomenclature 1992", the xylanase is classified as:

Generic name:	carbohydrase
IUB nomenclature:	endo-1,4- β -xylanase
IUB No.:	EC 3.2.1.8

Endo-1,4- β -xylanase hydrolyzes the β -(1,4)-D-xylosidic linkages in the arabinoxylan backbone. Arabinoxylans are highly branched xylans that are found in various cereals, and they exist both in a soluble and an insoluble form.

Identical xylanase genetic coding sequences were used in the construction of the production strains for the enzyme preparation subject to this evaluation (a *T. lanuginosus* xylanase expressed in a *F. venenatum* host strain) and an enzyme preparation, that was previously determined to be GRAS (a *T. lanuginosus* xylanase expressed in an *A. oryzae* host strain).

Substantial equivalence between the xylanase enzyme preparations was documented by characterization of the enzymes by means of SDS-PAGE, isoelectric focusing, crossed immunoelectrophoresis, and N-terminal amino acid sequencing. In addition, baking performance tests demonstrated that the enzyme preparations performed identically with respect to measured bread and dough parameters.

4. Manufacturing Process

The enzyme preparation is fermented, recovered and formulated according to the outline by Aunstrup et al. (1979, Reference D-1) and in accordance with current good manufacturing practices (cGMP). The quality management system used in the manufacturing process complies with ISO 9001.

The genetically modified strain of *F. venenatum*, carrying the *T. lanuginosus* xylanase gene, is grown by submerged fed-batch pure culture fermentation in a sterilized medium consisting of food or feed grade materials providing an adequate supply of nitrogen and carbon sources plus minerals and vitamins necessary for growth.

Raw materials used for fermentation and for recovery conform to Food Chemicals Codex (FCC) (1996, Reference B-9) specifications except those raw materials which do not appear in the FCC. For those not appearing in the FCC, internal specifications have been made in line with FCC requirements. All raw materials are quality controlled and subjected to the appropriate analyses to ensure their conformity with the specifications.

The production organism stock culture is controlled for absence of foreign microorganisms, viable count and enzyme generating ability. During fermentation, this control, as well as control of chemical and physical parameters to ensure the purity of the fermentation, are regularly performed.

The enzyme is recovered and purified from the culture broth by a series of concentration and filtration steps including a final germ filtration step, ensuring that no production organism is present.

The purified enzyme concentrate is finally standardized and stabilized with appropriate food grade ingredients. The product is further dried and granulated.

5. Composition and Specifications

The commercial enzyme preparation has the following formulation:

Enzyme solids (TOS*)	approx.	4%
Ash (mainly NaCl)	approx.	4%
Water	approx.	10%
Dextrin	approx.	4%
Sorbitol	approx.	1%
Wheat solids	approx.	77%

*TOS = Total Organic Solids, defined as: 100% - water - ash - diluents

The enzyme preparation complies with the purity criteria recommended for Enzyme Preparations as described by FCC (1996, Reference B-9). In addition, it also conforms to the General Specifications for Enzyme Preparations Used in Food Processing as proposed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (1992, Reference B-10).

Absence of the production organism is part of the complete specification. The manufacturing process includes a final germ filtration step to ensure that no production organism is present in the final enzyme concentrate.

6. Proposed Uses/Application

The active principle is endo-1,4- β -xylanase. The endo-1,4- β -xylanase hydrolyzes the β -(1,4)-D-xylosidic linkages in the arabinoxylan backbone. Arabinoxylans, that may have been described as pentosans in previous literature, are highly branched xylans that are characteristic for the outer cell walls and endosperm of cereals such as wheat, barley, rye, and oat. The backbone is β -(1,4) linked xylose which is mono or disubstituted with arabinose at the O-2 and/or O-3 position. Arabinoxylans exist in both soluble and insoluble forms.

Wheat flour contains 2-4% arabinoxylans; which play an essential role in bread making. The soluble fraction (approx. 1/3 of the amount) is essential for the water retention capacity of flour. The insoluble fraction is coupled to proteins and is believed to reduce the elasticity of the gluten complex.

The enzyme preparation is used in baking to improve handling and stability of the dough. By its action on the insoluble fraction of the arabinoxylans in flour, the elasticity of the gluten network is increased.

The enzyme preparation is used at minimum levels necessary to achieve the desired effect and according to requirements for normal production following cGMP. The recommended use level is 2-16 g per 100 kg flour corresponding to 50-400 Fungal Xylanase Units (FXU) per kg flour.

The enzyme is added to the flour and is active during the dough preparation and the leavening of the unbaked bread. During the baking process the high temperatures in the oven cause an inactivation of the enzyme activity.

Even though the enzyme activity is largely inactivated during the baking process, an estimation of human exposure is made, assuming that all enzyme activity is retained in the bread and that the enzyme preparation is utilized in all bread and related products at the highest recommended use level, in order to illustrate an exaggerated "worst case" situation.

The commercial enzyme preparation has an estimated organic solids content of 4% TOS and is used at levels up to 16 g per 100 kg flour. Using a standard recipe, 100 kg flour results in 140 kg bread, giving a theoretical content of 114 mg enzyme preparation/kg bread or 4.56 mg TOS/kg bread.

The average human intake of bread is estimated using well established statistics from various countries. Based on the highest daily intake of bread (158 g) the daily intake per person of the enzyme preparation would be 18.1 mg corresponding to (18.1 mg x 4%) TOS= 0.7 mg TOS per day. For a person weighing 60 kg this corresponds to 1.2×10^{-5} g TOS per kg body weight per day.

7. Biological Studies/Safety Evaluation

The following studies were performed on the test article, which is representative of the commercial enzyme preparation and is further described, including specifications, in the reference cited below.

- 13 weeks oral toxicity study in rats
- Test for mutagenic activity (Ames Test)
- Human lymphocyte cytogenetic assay

A paper summarizing the safety studies and evaluating the safety in use of the xylanase enzyme preparation produced by the genetically modified *F. venenatum* has been accepted for publication (Pedersen and Broadmeadow, 2000, Reference E-1).

The main conclusions of the safety studies were summarized as follows:

- Oral administration to rats of up to 10 ml of the test article per kg body weight per day (~ 89422 FXU/kg/day or 1.12 g TOS/kg/day) for 13 weeks reveals no signs of toxic effects related to treatment, and this dosage represents the No-Observed-Adverse-Effect-Level (NOAEL) in this study.
- The test article was not found to be genotoxic, as no mutagenic activity was observed in either the Ames' test or the human lymphocyte test.

Because the genetically modified *Fusarium venenatum* is considered a safe production strain for the xylanase enzyme preparation, the enzyme preparation is substantially equivalent to an enzyme preparation that was previously determined to be GRAS, the manufacturing process is in accordance with cGMPs, and the enzyme preparation meets the specifications and requirements outlined by FCC and JECFA, it is concluded that the xylanase enzyme preparation from *Fusarium venenatum*, expressing the gene encoding a xylanase from *Thermomyces lanuginosus*, is safe for use in the production of bread.

8. SUMMARY AND CONCLUSIONS

The guidelines for determining the safety of enzymes used in food processing (Pariza and Foster, 1983 and IFBC, 1990) were rigorously applied to a xylanase enzyme preparation produced by *Fusarium venenatum* expressing the gene encoding a xylanase from *Thermomyces lanuginosus* xylanase that was the basis of this critical evaluation. This enzyme is derived, using current good manufacturing practice, from a non-pathogenic and non-toxicogenic microorganism, which does not produce antibiotics and thus does not present a safety concern. In addition, this enzyme was reported to be non-genotoxic (negative in the Ames and human lymphocyte tests) and the NOAEL in a 13-week feeding study was 10 ml/kg bw/day (~1.12 g TOS/kg bw/day, the highest dose tested). The maximum anticipated daily intake of this enzyme (assuming that all bread and related products use this enzyme as a processing aid at the recommended level) would be 1.2×10^{-5} g TOS/kg bw/day.

We, the members of the Expert Panel, have critically evaluated the information summarized above and conclude that the xylanase enzyme preparation produced by *Fusarium venenatum* expressing the gene encoding a xylanase from *Thermomyces lanuginosus*, meeting appropriate food grade specifications and produced in accordance with current good manufacturing practices, is generally recognized as safe (GRAS), based on scientific procedures, for use as a processing aid in baking.

By:

Joseph F. Borzelleca, Chair

Timothy D. Phillips

Bruce B. Jarvis "

Robert H. Proctor

Michael W. Pariza

REFERENCES LISTS

- LIST A** *Curricula vitae* of members of the Expert Panel
- LIST B** General References
- LIST C** Production Strain References
- LIST D** Manufacturing Process Reference
- LIST E** Safety Reference

LIST A - *Curricula vitae* of members of the Expert Panel

1. Joseph F. Borzelleca, Chair
2. Bruce B. Jarvis
3. Michael W. Pariza
4. Timothy D. Phillips
5. Robert H. Proctor

000320

LIST B - General References

1. Pariza, M.W. and Foster, E.M.. Determining the Safety of Enzymes Used in Food Processing. *J. of Food Protection*, 46:5:453-468, 1983.
2. Berkowitz, D. and Maryanski, J.. Implications of biotechnology on international food standards and codes of practice. Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Eighteenth Session, Geneva, July 3-12, 1989.
3. IFBC (International Food Biotechnology Council). Chapter 4: Safety Evaluation of Foods and Food Ingredients Derived from Microorganisms in Biotechnologies and Food: Assuring the Safety of Foods Produced by Genetic Modification. *Regulatory Toxicology and Pharmacology* 12:S1-S196, 1990.
4. EU Scientific Committee for Food. Guidelines for the presentation of data on food enzymes. Reports of the Scientific Committee for Food, 27th series, 1991.
5. Organisation for Economic Cooperation and Development, Safety Evaluation of Foods Derived by Modern Biotechnology, 1993.
6. FAO/WHO. Biotechnology and Food Safety, Report of a Joint FAO/WHO Consultation. FAO Food and Nutrition Paper 61. Rome, Italy. 1996.
7. Jonas, D.A., Antignac, E., Antoine, J.M., Classen, H.G., Huggett, A., Knudsen, I., Mahler, J., Ockhuizen, T., Smith, M., Teuber, M., Walker, R., and de Vogel, P. The Safety Assessment of Novel Foods, Guidelines prepared by ILSI Europe Novel Food Task Force. *Food Chemical Toxicology* 34:931-940, 1996.
8. Organisation for Economic Cooperation and Development, Safety Considerations for Biotechnology, 1992.
9. Food Chemicals Codex, 4th Edition, National Academy of Sciences, Food and Nutrition Board, Committee on Food Chemicals Codex, National Academy Press, Washington, D.C., p. 133, 1996.
10. General Specifications for Enzyme Preparations Used in Food Processing, Joint FAO/WHO Expert Committee on Food Additives, Compendium of Food Additive Specifications, Vol. 1, Annex 1, FAO, 1992.

000321

LIST C - Production Strain References

1. Trinci, A.P.J., 1992. Myco-protein: A twenty-year overnight success story. *Mycol. Res.* 96:1-13.
2. O'Donnell, K. et al, 1998. Molecular Phylogenetic, Morphological, and Mycotoxin Data Support Reidentification of the Quorn Mycoprotein Fungus as *Fusarium venenatum*. *Fungal Genetics and Biology*, 23: 57-67.
3. Yoder, W.T. and Christianson, L.M., 1998. Species-Specific Primers Resolve Members of *Fusarium* Section *Fusarium*. Taxonomic Status Of the Edible "Quorn" Fungus Reevaluated. *Fungal Genetics and Biology*, 23: 69-80.
4. Nirenberg, H. 1995. Morphological differentiation of *Fusarium sambucinum* Fuckel sensu stricto, *F. torulosum* (Berk. & Curt.) Nirenberg comb. nov. and *F. venenatum* Nirenberg sp. nov. *Mycopathologia* 129: 131-141.
5. Royer, et al. 1995. *Fusarium graminearum* A 3/5 as a Novel Host for Heterologous Protein Production. *BIO/TECHNOLOGY* 13:1479-1483.
6. Yanish-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* 33:103-119.
7. Thompson et al, 1987. Characterization of the herbicide - resistance gene bar from *Streptomyces hygroscopicus*. *EMBO (Eur Mol Biol Org) J.* 6 (9): 2519-2523).
8. Corrick, C. M., A. P. Twomey, and M. J. Hynes. The nucleotide sequence of the *amdS* gene of *Aspergillus nidulans* and the molecular characterization of 5' mutations. *Gene* 53:63-71. 1987.
9. Boel, E. et al. 1984. Two Different Types of Intervening Sequences in the Glucoamylase Gene from *Aspergillus niger*. *EMBO* 3:1581-1585.
10. Royer, J.C. et al, 1999. Deletion of the Trichodiene Synthase Gene of the Fungus *Fusarium venenatum*; Two Systems for Repeated Gene Deletions. *Fungal Genetics and Biology*, 28: 68-78.
11. Miller, J. D. and MacKenzie, S., 2000. Secondary metabolites of *Fusarium venenatum* strains with deletions in the *Tri5* gene encoding trichodiene synthetase. *Mycologia*. In Press.

000322

LIST D - Manufacturing Process Reference

1. Aunstrup et al: Production of Microbial Enzymes, Microbial Technology, Academic Press, 1979, 2nd Edition, Vol. 1, 281-309.

LIST E - Safety Reference

1. Pedersen, P. B. and Broadmeadow, A., 2000. Safety evaluation of the *Thermomyces lanuginosus* xylanase expressed by *Fusarium venenatum*, intended for use in food. Food Additives & Contaminants. In Press.

000323

List
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Personal History

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 Richmond, VA 23298-0613

Educational Background

B.S.	St. Joseph's University, Philadelphia, PA Biology, Chemistry	1952
M.S.	School of Graduate Studies Thomas Jefferson University Jefferson Medical College, Philadelphia, PA Pharmacology, Physiology	1954
Ph.D.	School of Graduate Studies Thomas Jefferson University Jefferson Medical College, Philadelphia, PA. Pharmacology, Biochemistry	1956

Academic Appointments

Department of Pharmacology Medical College of Pennsylvania Philadelphia, PA	Instructor-Associate	1956-1959
Department of Pharmacology Toxicology Medical College of Virginia Richmond, VA 23298-0613	Assistant Professor Associate Professor Professor Head, Division of Toxicology Professor Emeritus, Pharmacology & Toxicology	1959-1962 and 1962-1967 1967- 1972-1986 01 July 1996-

Professional Certification

Fellow, Academy of Toxicological Sciences

Professional Affiliations

Societies

Academy of Toxicological Sciences**
 American Association for the Advancement of Science
 American Chemical Society
 American College of Toxicology*
 American Society of Pharmacology and Experimental Therapeutics**
 (Environmental Pharmacology Committee; Liaison Committee, SOT; Toxicology
 Committee)
 International Society of Regulatory Toxicology and Pharmacology*
 (Member of Council)
 Sigma XI
 Society of Experimental Biology and Medicine*
 (Councilor; Program Chairman of Southeastern Section)
 Society for Risk Analysis
 Society of Toxicology* **
 (Member and/or Chairman: Awards, Education, Legislative Affairs, Membership,
 Nominating Committees; Secretary of the Society, Councilor, and President; President,
 Food Safety Specialty Section)
 Virginia Academy of Science*
 (Chairman, Medical Sciences Division)

- Held elected office
- ** Held appointed office or position

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Journals

Editor, Food Chemical Toxicology, 1992-

Editorial Board

Environmental Carcinogenesis Reviews, 1981-
 Journal of Environmental Pathology, Toxicology and Oncology 1977-
 Journal of Environmental Science and Health, 1979-
 Journal of the American College of Toxicology, 1982-
 Journal of Toxicology: Cutaneous and Ocular Toxicology, 1982- 1992
 Journal of Applied Toxicology, 1989-
 Pharmacology, 1978-
 Pharmacology and Drug Development, 1980-
 Toxicology and Applied Pharmacology, 1975-1978

Consultantships (Past, Present)**Governmental**

Food and Drug Administration
 National Institute of Mental Health
 National Cancer Institute
 Environmental Protection Agency
 Department of Labor - OSHA (Chairman, Carcinogens Standards Committee)
 U.S. Army - Research and Development Command

Non-Governmental

National Academy of Sciences - NRC
 Committee on Toxicology (Member, Chairman)/Board on Toxicology and Environmental Health Hazards
 Safe Drinking Water Committee
 Evaluation of Household Substances Committee (1138 Committee)
 Food Protection Committee
 Food Additives Survey Committee
 Committee on Risk-Based Criteria for Non-RCRA Hazardous Wastes
 Committee on Risk Assessment of Flame-Retardant Chemicals

Federation of American Societies of Experimental Biology
 Select Committee on GRAS Substances
 Flavors and Extracts
 Biotechnology Product Safety
 Caprenin GRAS Committee

World Health Organization
 Joint Meeting on Pesticide Residues (JMPR) (Member, Chairman)

Industrial

Chemical Companies; Trade Associations

University Activities**Related to Instruction**

Prepared a laboratory manual in pharmacology (animal and human studies) (1960)
 Introduced the use of closed circuit TV and TV tapes in pharmacology (1960)
 Introduced clinical pharmacological experiments into the medical and dental programs (1960)
 Planning and participation in continuing education program
 (Schools of Dentistry, Medicine and Pharmacy)
 Planning and administering each of the three major efforts in pharmacology
 (dental, medical, pharmacy) since 1960.
 Graduate Program - assisted in developing graduate training program in toxicology

Current Teaching Activities

Present lectures in the following: INH 511/512; PMC 535, 536, 539; PHA 591; MPH 604; PIO (MPH V) 691; PMC 400, 404; PMC 609; Dietetic Intern Program; M-I;
 Biology; Nutrition electives

Honors

Not Directly Related to Instruction

Elected senator from the graduate school, then vice-president of the University Senate
 Served on various committees (e.g. Curriculum, Search, Animal Care,) in each of the four major schools (Dentistry, Graduate, Medical, Pharmacy)

Research

Research has been continuously funded since 1956. Sources of support include governmental (U.S.P.H.S.; N.I.H; E.P.A.; N.I.D.A.) and non-governmental (industrial). (A list of publications is attached).

Awards

DOD - US Army - Chemical Research Development and Engineering Center
Distinguished Service Award, 1986

National Italian - American Foundation Award
Excellence in Medicine and Community Service, 1987

Thomas Jefferson University
Distinguished Alumnus Award, 1987

Virginia Commonwealth University - School of Basic Health Sciences
Outstanding Faculty Award, 1987

Virginia Commonwealth University - School of Basic Health Sciences, Dept. of
Pharmacology and Toxicology
Professor of the Year- 1992

American College of Toxicology
Distinguished Service Award- 1997

PUBLICATIONS

Borzelleca, J.F. and Manthei, R.W.: Factors influencing pentobarbital sleeping time in mice. Arch. Int. Pharmacodyn. 111: 296, 1957.

Borzelleca, J.F.: Studies of the contribution of bladder absorption to the physiological changes induced by pentobarbital. J. Pharm. Exp. Ther. 129: 305, 1960.

Borzelleca, J.F.: The absorption of nicotine from the urinary bladder of the dog. Arch. Int. Pharmacodyn. 133: 444, 1961.

Borzelleca, J.F., Bowman, E.R. and McKennis, H., Jr.: The cardiovascular and respiratory effects of (-)-cotinine. J. Pharmacol. Exp. Ther. 137: 313, 1962.

Borzelleca, J.F.: Drug absorption from the urinary tract of the rat. Nicotine. Arch. Int. Pharmacodyn. 143: 595, 1963.

Borzelleca, J.F.: Influence of saline and glucose infusions on the course of barbiturate intoxication. Arch. Int. Pharmacodyn. 146: 163, 1963.

Larson, P.S., Borzelleca, J.F., Bowman, E.R., Crawford, E.M., Smith, R.B., Jr. and Henningar, G.R.: Toxicologic studies on a preparation of p-tertiary octylphenoxy-polyethoxy ethanols (Triton X-405). Toxicol. Appl. Pharmacol. 5: 782, 1963.

Borzelleca, J.F., Larson, P.S., Henningar, G.R., Hug, E.G., Crawford, E.M. and Smith, R.B., Jr.: Studies on the chronic oral toxicity of monomeric ethyl acrylate and methyl methacrylate. Toxicol. Appl. Pharmacol. 6: 29, 1964.

Borzelleca, J.F. and Cherrick, H.: The excretion of drugs in saliva. Antibiotics. J. Oral Therap. Pharmacol. 2: 180, 1965.

Borzelleca, J.F. and Lester, D.: Acute toxicity of some perhalogenated acetones. Toxicol. Appl. Pharmacol. 7: 592, 1965.

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PUBLICATIONS

Borzelleca, J.F.: Drug movement from the isolated urinary bladder of the rabbit. Arch. Int. Pharmacodyn. 154: 40, 1965.

Borzelleca, J.F.: Rabbit urinary bladder potentials. Invest. Urol. 3: 77, 1965.

Borzelleca, J.F.: Studies on the mechanisms of drug movement from the isolated urinary bladder. J. Pharmacol. Exp. Ther. 148: 111, 1965.

Lowenthal, W. and Borzelleca, J.F.: Drug absorption from the rectum. I. J. Pharm. Sci. 54: 1790, 1965.

Ambrose, A.M., Borzelleca, J.F., Larson, P.S., Smith, R.B., Jr. and Hennigar, G.R.: Toxicologic studies on monochloroacetaldehyde: 2,4-dinitrophenylhydrazone, a foliar fungicide. Toxicol. Appl. Pharmacol. 8: 472, 1966.

Borzelleca, J.F. and Doyle, C.H.: Excretion of drugs in saliva. Salicylate, barbiturate, sulfanilamide. J. Oral Therap. Pharmacol. 3: 104, 1966.

Borzelleca, J.F. and Lowenthal, W.: Drug absorption from the rectum. II. J. Pharm. Sci. 55: 151, 1966.

Wooles, W.R. and Borzelleca, J.F.: Prolongation of barbiturate sleeping time in mice by stimulation of the reticuloendothelial system. J. Reticuloendo. Soc. 3: 41, 1966.

Wooles, W.R., Borzelleca, J.F. and Branham, G.W.: The effects of acute and prolonged salicylate administration on liver and plasma triglyceride levels and dietary-induced hypercholesterolemia. Toxicol. Appl. Pharmacol. 10: 1, 1967.

Borzelleca, J.F., Harris, T. and Bernstein, S.: The effect of DMSO on drug movement through the wall of the urinary bladder of the rabbit. J. Invest. Urol. 6: 43, 1968.

Borzelleca, J.F.: The excretion of glucose in saliva. Dog. J. Oral Therap. Pharmacol. 4: 338, 1968.

Kim, K.S., Borzelleca, J.F., McKennis, H. and Bowman, E.R.: Pharmacological effects of some nicotine metabolites and related compounds. J. Pharmacol. Exp. Ther. 161: 59, 1968.

Marcus, S. and Borzelleca, J.F.: Observations of reserpine-induced bradycardia. Arch. Int. Pharmacodyn. 174: 12, 1968.

Schwartz, S.L. and Borzelleca, J.F.: Adrenergic blood pressure response in the shark. Science 163: 395, 1969.

Ambrose, A.M., Borzelleca, J.F., Larson, P.S. and Hennigar, G.R.: The toxicology of a foliar fungicide, GC-4072. Toxicol. Appl. Pharmacol. 17: 323, 1970.

Borzelleca, J.F. and Putney, J.W., Jr.: A model for the movement of salicylate across the parotid epithelium. J. Pharmacol. Exp. Ther. 174: 527, 1970.

Borzelleca, J.F. and Putney, J.W., Jr.: Studies on the biotransformation of salicylic acid by the salivary gland. Arch. Int. Pharmacodyn. 188: 127, 1970.

Lowenthal, W., Borzelleca, J.F. and Corder, C.D., Jr.: Drug absorption from the rectum. III. Aspirin and some aspirin derivatives. J. Pharm. Sci. 59: 1353, 1970.

Putney, J.W., Jr. and Borzelleca, J.F.: A method for the determination of small quantities of salicylate metabolites in the presence of a great excess of salicylic acid. Arch. Int. Pharmacodyn. 188: 119, 1970.

Wynn, J.E., van't Riet, B. and Borzelleca, J.F.: Excretion and toxicity of EGTA and EDTA after oral administration to rats. Toxicol. Appl. Pharmacol. 16: 807, 1970.

PUBLICATIONS

- Ambrose, A.M., Larson, P.S., Borzelleca, J.F., Smith, R.B., Jr. and Hennigar, G.R.: Toxicologic studies on 2,4-dichlorophenyl-p-nitrophenyl ether. *Toxicol. Appl. Pharmacol.* 19: 263, 1971.
- Borzelleca, J.F., Larson, P.S., Crawford, E.M., Hennigar, G.R., Jr., Kuchar, E.J. and Klein, H.H.: Toxicologic and metabolism studies on pentachloronitrobenzene. *Toxicol. Appl. Pharmacol.* 18: 522, 1971.
- Putney, J.W., Jr. and Borzelleca, J.F.: On the mechanisms of ¹⁴C-salicylic acid distribution in rat submaxillary gland in vitro. *J. Pharmacol. Exp. Ther.* 117: 263, 1971.
- Putney, J.W., Jr. and Borzelleca, J.F.: On the mechanisms of ¹⁴C-nicotine distribution in rat submaxillary gland in vitro. *J. Pharmacol. Exp. Ther.* 178: 180, 1971.
- Ambrose, A.M., Larson, P.S., Borzelleca, J.F. and Hennigar, G.R.: Toxicologic studies on 3',4'-dichloropropionanilide. *Toxicol. Appl. Pharmacol.* 23: 650, 1972.
- Egle, J.L., Jr., Putney, J.W., Jr. and Borzelleca, J.F.: Cardiac rate and rhythm in mice affected by haloalkane propellants. *J.A.M.A.* 222: 786, 1972.
- Putney, J.W., Jr. and Borzelleca, J.F.: On the mechanisms of ¹⁴C-salicylic acid excretion by the rat submaxillary gland. *J. Pharmacol. Exp. Ther.* 182: 515, 1972.
- Putney, J.W., Jr. and Borzelleca, J.F.: Active accumulation of ¹⁴C-salicylic acid by rat kidney cortex *in vitro*. *J. Pharmacol. Exp. Ther.* 186: 600, 1973.
- Borzelleca, J.F.: Safety evaluation and toxicological tests and procedures. *J.A.O.A.C.* 58: 692, 1975.
- Adams, M.D., Wedig, J.H., Jordan, R.L., Smith, L.W., Henderson, R. and Borzelleca, J.F.: Urinary excretion and metabolism of salts of 2-pyridinethiol-1-oxide following intravenous administration to female yorkshire pigs. *Toxicol. Appl. Pharmacol.* 36: 523, 1976.
- Allen, M.A., Wrenn, J.M., Putney, J.W., Jr. and Borzelleca, J.F.: A study of the mechanism of transport of diphenylhydantoin in the rat submaxillary gland *in vitro*. *J. Pharmacol. Exp. Ther.* 197: 408, 1976.
- Ambrose, A.M., Larson, P.S., Borzelleca, J.F. and Hennigar, G.R.: Long-term toxicologic assessment of nickel in rats and dogs. *J. Food Science and Technology* 13: 181, 1976.
- Egle, J.L., Jr., Long, J.E., Simon, G.S. and Borzelleca, J.F.: An evaluation of the cardiac sensitizing potential of a fabric protector in aerosol form, containing 1,1,1-trichloroethane. *Toxicol. Appl. Pharmacol.* 38: 369, 1976.
- EGLE, J.L., Jr., Fernandez, S.B., Guzelian, P.S. and Borzelleca, J.F.: Distribution and excretion of chlordecone (Kepone) in the rat. *Drug Metab. Dispos.* 6:91, 1976
- Munson, A.E., Barrett, B.A. and Borzelleca, J. F.: *In vitro* experimental approaches to detection of sensitive agents. In: *Cutaneous Toxicity*, (V. Drill, ed.), Academic Press, Inc., San Francisco, p. 175, 1977.
- Weinberg, A.D., Dimen, E.M., Borzelleca, J.F. and Harris, L.S.: Weight and activity in male mice after daily inhalation of cannabis smoke in an automated smoke exposure chamber. *J. Pharm. & Pharmac.* 29: 477, 1977.
- Weinberg, A.D., Dimen, E.M., Simon, G.S., Harris, L.S. and Borzelleca, J.F.: Measurements of weight and activity in male mice following inhalation of cannabis smoke in a controlled smoke exposure chamber. *Toxicol. Appl. Pharmacol.* 42: 301, 1977.
- Allen, M.A., Wrenn, J.M., Putney, J.W., Jr. and Borzelleca, J.F.: A study of the mechanisms of transport of benzylpenicillin in the rat submaxillary gland. *Arch. Int. Pharmacodyn.* 233: 180, 1978.

PUBLICATIONS

- Bowman, F.J., Borzelleca, J.F. and Munson, A.E.: The toxicity of some halomethanes in mice. *Toxicol. Appl. Pharmacol.* **44**: 213, 1978.
- Egle, J.L., Jr., Fernandez, S.B., Guzelian, P.S. and Borzelleca, J.F.: Distribution and excretion of chlordecone (Kepone) in the rat. *Drug Metab. Dispos.* **6**: 91, 1978.
- McConnell, W.R. and Borzelleca, J.F.: A study of the mechanism of transport of Δ^9 -tetrahydrocannabinol in the rat submaxillary gland *in vivo*. *Arch. Int. Pharmacodyn* **235**: 180, 1978.
- McConnell, W.R., Dewey, W.L., Harris, L.S. and Borzelleca, J.F.: A study of the effect of delta-9-tetrahydrocannabinol (delta-9-THC) on mammalian salivary flow. *J. Pharmacol. Exp. Ther.* **206**: 567, 1978.
- Schumann, A.M. and Borzelleca, J.F.: An assessment of the methemoglobin and Heinz body inducing capacity of pentachloronitrobenzene in the cat. *Toxicol. Appl. Pharmacol.* **44**: 523, 1978.
- Simon, G.S., Tardiff, R.G. and Borzelleca, J.F.: Potential mutagenic and adverse male reproductive effects of 1,2,3,4-tetrabromobutane. A dominant lethal study in the rat. *Toxicol. Appl. Pharmacol.* **44**: 661, 1978.
- Carmines, E.L., Carchman, R.A. and Borzelleca, J.F.: Kepone: Cellular sites of action. *Toxicol. Appl. Pharmacol.* **49**: 543, 1979.
- Egle, J.L., Jr., Guzelian, P.S. and Borzelleca, J.F.: Time course of the acute toxic effects of sublethal doses of chlordecone (Kepone). *Toxicol. Appl. Pharmacol.* **48**: 533, 1979.
- Larson, P.S., Egle, J.L., Jr., Hennigar, G.R. and Borzelleca, J.F.: Acute and subchronic toxicity of mirex in the rat, dog, and rabbit. *Toxicol. Appl. Pharmacol.* **49**: 271, 1979.
- Larson, P.S., Egle, J.L., Jr., Hennigar, G.R., Lane, R.W. and Borzelleca, J.F.: Acute, subchronic and chronic toxicity of chlordecone. *Toxicol. Appl. Pharmacol.* **48**: 29, 1979.
- Simon, G.S., Kuchar, E.J., Klein, H.H. and Borzelleca, J.F.: Distribution and clearance of pentachloronitrobenzene in chickens. *Toxicol. Appl. Pharmacol.* **50**: 401, 1979.
- Simon, G.S., Tardiff, R.G. and Borzelleca, J.F.: Failure of hexachlorobenzene to induce dominant lethal mutations in the rat. *Toxicol. Appl. Pharmacol.* **47**: 415, 1979.
- Borzelleca, J.F. and Skalsky, H.L.: The excretion of pesticides in saliva and its value in assessing exposure. *J. Environ. Sci. Health*, B15(6), 843, 1980.
- Borzelleca, J.F., Egle, J.L., Jr., Hennigar, G.R., Klein, H.H., Kuchar, E.J., Lane, R.W. and Larson, P.S.: A toxicologic evaluation of 5-ethoxy-3-trichloromethyl-1,2,4-triazole (ETMT). *Toxicol. Appl. Pharmacol.* **56**: 164, 1980.
- Carmines, E.L., Carchman, R.A. and Borzelleca, J.F.: A method for the evaluation of dose-effect data utilizing a programmable calculator. *J. Environ. Path. and Tox.* **4**: 23, 1980.
- Kessler, F.K., Laskin, D.L., Borzelleca, J.F. and Carchman, R.A.: Assessment of somatogenotoxicity of povidone-iodine using two *in vitro* assays. *J. Environ. Path. and Tox.* **3**: 327, 1980.
- Skalsky, H.L., Wrenn, J.M. and Borzelleca, J.F.: *In vitro* and *in vivo* evaluation of the movement of Kepone in the rat submaxillary gland. *J. Environ. Path. and Tox.* **3**: 529, 1980.
- Smith, L.W. and Borzelleca, J.F.: Excretion of cadmium and mercury in rat saliva. *Toxicol. Appl. Pharmacol.* **54**: 134, 1980.
- Smith, L.W. and Borzelleca, J.F.: *In vitro* stimulation of oxygen consumption in rat submaxillary gland by pilocarpine. *J. Dent. Res.* (59)9: 1531, 1980.

PUBLICATIONS

- Smith, L.W. and Borzelleca, J.F.: Movement of cadmium in rat submaxillary slices. *Toxicol. Appl. Pharmacol.* 55: 403, 1980.
- Smith, L.W. and Borzelleca, J.F.: Movement of mercury in rat submaxillary slices. *Toxicology* 18: 169, 1980.
- Borzelleca, J.F.: Report of the NATO/CCMS drinking water pilot study on health aspects of drinking water contaminants. *Sci. of the Total Environ.* 18: 205, 1981.
- Carmines, E.L., Carchman, R.A. and Borzelleca, J.F.: Investigations into the mechanism of paraquat toxicity utilizing a cell culture system. *Toxicol. Appl. Pharmacol.* 58: 353, 1981.
- Simon, G.S., Borzelleca, J.F. and Dewey, W.L.: Narcotics and diabetes II. Streptozotocin-induced diabetes selectively alters the potency of certain narcotic analgesics. Mechanism of diabetes: morphine interaction. *J. Pharmacol. Exp. Ther.* 218: 324, 1981.
- Balster, R.L. and Borzelleca, J.F. The behavioral toxicity of trihalomethane contaminants Of drinking water in mice. *Environ. Health Perspec.* 46: 127, 1982.
- Kauffmann, B.M., White, K.L., Jr., Sanders, V.M., Douglas, K.A., Sain, L.E., Borzelleca, J.F. and Munson A.E.: Humoral and cell-mediated immune status in mice exposed to chloral hydrate. *Environ. Health Perspec.* 44: 147, 1982.
- Lane, R.W., Riddle, B.L. and Borzelleca, J.F.: Effects of 1,2-dichloroethane and 1,1,1-trichloroethane in drinking water on reproduction and development in mice. *Toxicol. Appl. Pharmacol.* 63: 409, 1982.
- Munson, A.E., Sain, L.E., Sanders, V.M., Kauffmann, B.M., White, K.L., Jr., Page, D.G., Barnes, D.W., and Borzelleca, J.F.: Toxicology of organic drinking water contaminants: trichloromethane, bromodichloromethane, dibromochloromethane and tribromomethane. *Environ. Health Perspec.* 46: 117, 1982.
- Sanders, V.M., Kauffmann, B.M., White, K.L., Douglas, K.A., Barnes, D.W., Sain, L.E., Bradshaw, T.J., Borzelleca, J.F. and Munson, A.E.: Toxicology of chloral hydrate in the mouse. *Environ. Health Perspec.* 44: 137, 1982.
- Sanders, V.M., Tucker, A.N., White, K.L., Jr., Kauffmann, B.M., Hallett, P., Carchman, R.A., Borzelleca, J.F. and Munson, A.E.: Humoral and cell-mediated immune status in mice exposed to trichloroethylene in the drinking water. *Toxicol. Appl. Pharmacol.* 62: 358, 1982.
- Borzelleca, J.F.: A review of volatile organic contaminant data. *Proc. AWWA Water Quality Tech. Conf.* 225, 1983.
- Charles, J.L., Kram, D., Borzelleca, J.F. and Carchman, R.A.: The kinetics of *in vivo* sister chromatid exchange induction in mouse bone marrow cells by alkylating agents. I. Cyclophosphamide. *Environ. Mut.* 5: 825, 1983.
- Borzelleca, J.F., Condie, L.W. and Hayes, J.R.: Toxicological evaluation of selected chlorinated phenols. *Proceedings of the 5th International Water Disinfection Conference, Williamsburg, VA, 1984*.
- Borzelleca, J.F.: Food safety: regulations, research, and results. *Va. Med.* 111: 390, 1984.
- Seyler, D.E., East, J.M., Condie, L.W. and Borzelleca, J.F.: The use of *in vitro* methods for assessing reproductive toxicity of dichlorophenols. *Tox. Letters* 20: 309, 1984.
- Shopp, G.M., White, K.L., Jr., Holsapple, M.P., Barnes, D.W., Duke, S.S., Anderson, A.C., Condie, L.W., Jr., Hayes, J.R. and Borzelleca, J.F.: Naphthalene toxicity in CD-1 mice: general toxicology and immunotoxicology. *Fund. Appl. Toxicol.* 4: 406, 1984.

PUBLICATIONS

- Borzelleca, J.F. and Hogan, G.K.: Chronic toxicity/carcinogenicity study of FD&C Blue No. 2 in mice. *Food Chem. Tox.* 23: 719, 1985.
- Borzelleca, J.F., Hayes, J.R., Condie, L.W. and Egle, J.L., Jr.: Acute toxicity of monochlorophenols, dichlorophenols and pentachlorophenol in the mouse. *Toxicol. Letters* 29: 39, 1985.
- Borzelleca, J.F., Hayes, J.R., Condie, L.W. and Egle, J.L.: Acute and subchronic toxicity of 2,4-dichlorophenol in CD-1 mice. *Fund. Appl. Toxicol.* 5: 478, 1985.
- Borzelleca, J.F., Hogan, G.K. and Koestner A.: Chronic toxicity/carcinogenicity study of FD&C Blue No. 2 in rats. *Food Chem. Tox.* 23: 551, 1985.
- Hayes, J.R. and Borzelleca, J.F.: Nutrient interaction with drugs and other xenobiotics, *J. Am. Dietetic Assoc.* 85: 3 335, 1985.
- Lane, R.W., Simon, Glen, S.S., Dougherty, R.W., Egle, J.L. and Borzelleca, J.F.: Reproductive toxicity and lack of dominant lethal effects of 2,4-dinitrotoluene in the male rat. *Drug and Chem. Tox.* 4: 265, 1985.
- Borzelleca, J.F., Goldenthal, E.I. and Wazeter, F.X.: A multigeneration study of FD&C Blue No. 2 in rats. *Food Chem. Tox.* 24: 159, 1986.
- Charles, J.L., Jacobson-Kram, D., Condie, L.W., Jr., Borzelleca, J.F. and Carchman, R. A.: The kinetics of *in vitro* sister chromatid exchange induction in mouse bone marrow cells by ethylnitrosourea and methylnitrosourea. *Toxicol. Appl. Pharmacol.* 84: 56, 1986.
- Hayes, J.R., Condie, L.W., Jr. and Borzelleca J.F.: The subchronic toxicity of tetrachorethylene (perchloroethylene) administered in the drinking water of rats. *Fund. Appl. Toxicol.* 7: 119, 1986.
- Hayes, J.R., Condie, L.W., Jr. and Borzelleca, J.F.: Acute, 14-day repeated dosing and 90-day subchronic toxicity studies of carbon tetrachloride in CD-1 mice. *Fund. Appl. Toxicol.* 7: 454, 1986.
- Hayes, J.R., Condie, L.W., Jr. and Borzelleca, J.F.: Acute, 14-day repeated dosing, and 90-day subchronic toxicity studies of potassium picloram. *Fund. Appl. Toxicol.* 7: 464, 1986.
- Hayes, J.R., Condie, L.W., Jr. and Borzelleca, J.F.: Toxicology of haloacetoneitriles. *Environ. Health Perspec.* 69: 183, 1986.
- Lane, R.W., Sturm, R.J., Borzelleca, J.F. and Carchman, R.A.: Effect of *in vitro* differentiation on phorbol diester receptor number in human promyelocytic leukemia (HL-60) cells. *Cancer Res.* 46: 3782, 1986.
- Simon, G.S., Egle, J.L., Jr., Dougherty, R.W. and Borzelleca, J.F.: Dominant lethal assay of chlordecone and its distribution in the male reproductive tissues of the rat. *Tox. Letters* 30: 237, 1986.
- Tarka, S.M., Jr., Applebaum, R.S. and Borzelleca, J.F.: Evaluation of the perinatal, postnatal and teratogenic effects of coca powder and theobromine in Sprague-Dawley/CD rats. *Food Chem. Tox.* 24: 375, 1986.
- Tarka, S.M., Jr., Applebaum, R.S. and Borzelleca, J.F.: Evaluation of the teratogenic potential of cocoa powder and theobromine in New Zealand white rabbits. *Food Chem. Tox.* 24: 363, 1986.
- Borzelleca, J.F., Capen, C.C. and Hallagan, J.B.: Lifetime toxicity/carcinogenicity study of FD&C Red no. 3 (erythrosine) in rats. *Fd. Chem. Toxic.* 25: 723, 1987.

PUBLICATIONS

Borzelleca, J.F., Capen, C.C., and Hallagan, J.B.: Lifetime toxicity/carcinogenicity study of FD&C Red No. 3 (erythrosine) in mice. *Fd. Chem. Toxic.* 25: 735, 1987.

Hayes, J.R., Condie, L.W., Jr., Egle, J.L., Jr. and Borzelleca, J.F.: The acute and subchronic toxicity in rats of trans-1,2 dichloroethylene in drinking water. *J.Am. Coll. Toxicol.* 6: 471, 1987.

Borzelleca, J.F. and Hallagan, J.B.: Chronic toxicity/carcinogenicity studies of FD&C Yellow No. 5 (tartrazine) in rats. *Fd. Chem. Toxic.* 26: 179, 1988.

Borzelleca, J.F., Condie, L.W., Jr. and Egle, J.L.: Short-term toxicity (one-and ten-day gavage) of barium chloride in male and female rats. *J. Am. Coll. Toxicol.* 7: 675-685, 1988.

Condie, L.W., Jr., Hill, J.R. and Borzelleca, J.F.: Oral toxicology studies with xylene isomers and mixed xylenes. *Drug and Chem. Tox.* 11: 329, 1988.

Borzelleca, J.F. and Hallagan, J.B.: A chronic toxicity/carcinogenicity study of FD&C yellow no. 5 (tartrazine) in mice. *Fd. Chem. Toxic.* 26:189, 1988.

Borzelleca, J.F., Clark, E.C. and Condie, L.W., Jr.: Short-term toxicity (1 and 10 days) of cadmium chloride in male and female rats: gavage and drinking water. *J. Am. Coll. Toxicol.* 8: 377, 1989.

Borzelleca, J.F., Condie, L.W., Jr., Clarke, E.C. and Egle, J.L.: Short-term toxicity (one and ten day gavage) of potassium dichromate in male and female rats. *J. Am. Coll. Toxicol.* 8: 1197, 1989.

Borzelleca, J.F., Olson, J.W.A. and Reno, F.A.: Lifetime toxicity/carcinogenicity study of FD&C red No. 40 (allura red) in Sprague-Dawley rats. *Fd. Chem. Tox.* 27: 701, 1989.

Borzelleca, J.F.: Status of colors and flavors used in the confectionery industry. *Proc. 106th Annual Convention of the National Confectioners Association of the United States.* 33, 1989.

Lamb, R.G., Borzelleca, J.F., Condie, L.W. and Gennings, C.: Toxic interactions between carbon tetrachloride and chloroform in cultured rat hepatocytes. *Toxicol. Appl. Pharmacol.* 101: 106, 1989.

O'Hara, T.M., Borzelleca, J.F., Clark, E.C., Sheppard, M.A. and Condie, L.W., Jr.: A CCl₄/CHCl₃ interaction study in isolated hepatocytes: selection of a vehicle. *Fund. Appl. Toxicol.* 13: 605, 1989.

Borzelleca, J.F. and Hallagan, J.B.: Multigeneration study of FD&C red no. 3 (erythrosine) in sprague-dawley rats. *Fd. Chem. Tox.* 28:813, 1990

Borzelleca, J.F., Depukat, K. and Hallagan, J.B.: Lifetime toxicity/carcinogenicity studies of FD&C blue no. 1 (brilliant blue FCF) in rats and mice. *Fd. Chem. Toxic.* 28:221,1990.

Borzelleca, J.F., O'Hara, T.M., Gennings, C., Granger, R.H., Sheppard, M.A. and Condie, L.W., Jr.: Interactions of water contaminants. I. Plasma enzyme activity and response surface methodology following gavage administration of CCl₄ and CHCl₃ or TCE singly and in combination in the rat. *Fund. Appl. Toxicol.* 14: 477, 1990.

Borzelleca, J.F., Olson, J.W.A. and Reno, F.A.: Lifetime toxicity/carcinogenicity study of FD&C red no. 40 (allura red) in mice. *Fd. Chem. Tox.* 29:313, 1991.

O'Hara, T.M., Sheppard, M.A., Clarke, E.C., Borzelleca J.F., Gennings, C. and Condie, L.W., Jr.: A CCl₄/CHCl₃ interaction study in isolated hepatocytes: non-induced, and phenobarbital pretreated cells. *J.Appl. Toxicol.* 11:147, 1991

PUBLICATIONS

- Borzelleca, J.F.: Assessment of Safety/Risk of Chemicals- Inception and Evolution of the ADI and Dose-Response Modeling Procedures- Commentary. *Tox. Letters* 59:1, 1991
- Borzelleca, J.F.: The safety evaluation of macronutrient substitutes. *CRC Critical Reviews in Food Science and Nutrition*. 32:127, 1992
- Borzelleca, J.F.: Macronutrient Substitutes: Safety Evaluation. *Reg. Tox. Pharm.* 16: 253, 1992
- Waddell, W.J., Borzelleca, J.F., Doull, J., Grasso, P., LeBourhis, B., Levy, P.S. and Tamburro, C.H. *Alcohol and Cancer*. *Br. J. Cancer*. 66:1200, 1992
- Borzelleca, J.F.: Evaluation of the safety of tara gum as a food ingredient: a review of the literature. *J. Am. Coll. Tox.* 12 (1):81,1993
- Borzelleca, J.F. and Egle, J. L. Jr.: An evaluation of the reproductive and developmental effects of tara gum in rats. *J. Am. Coll. Tox.* 12 (1): 91, 1993
- Borzelleca, J.F.: Interactions of environmental chemicals and toxins in Proceedings of the Second Princess Chulabhorn Science Congress: "Environment, Science and Technology: the Challenges of the 21st Century." 1993
- Borzelleca, J.F., Egle, J.L., Jr., Harris, L.S., Johnson, D.N., Terrill, J.B. and Belleville, J.A.N.: Toxicological Evaluation of u-Agonists Part I. Assessment of Toxicity Following 30 Days of Repeated Oral Dosing of Male and Female Rats with Levo-Alpha-Acetylmethadol HCl (LAAM). *J. Appl. Tox.* 14 (6): 435, 1994
- Conn, R.E., Kolstad, J.J., Borzelleca, J.F., Dixler, D.S., Filer, L.J., Jr, LaDu, B.N., Jr, and Pariza, M.W.: Safety Assessment of Polylactide (PLA) for Use as a Food-contact Polymer. *Fd. Chem. Tox.* 33:273-283, 1995
- Hallagan, J.B., Allen, D.C., and Borzelleca, J.F.: The safety and regulatory status of food, drug and cosmetics color additives exempt from certification. *Fd. Chem. Toxic.* 33:515, 1995
- Borzelleca, J.F.: Post-Marketing Surveillance of Macronutrient Substitutes. *Fd. Tech.*49:107-113, 1995
- Borzelleca, J.F., Egle, J.L., Jr., Harris, L.S. and Belleville, J.A.N.: Toxicological Evaluation of u-Agonists. Part II: Assessment of toxicity Following 30 Days of Repeated Oral Dosing of Male and Female Rats with Levo-alpha-noracetylmethadol HCl (NorLAAM). *J. Appl. Tox.* 15(5): 339-355, 1995
- Moore, K.A., Lichtman, A.H., Poklis, A., and Borzelleca, J.F.: alpha-Benzyl-N-methylphenethylamine (BNMPA), an impurity of illicit methamphetamine synthesis: pharmacological evaluation and interaction with methamphetamine. *Drug and Alcohol Dependence* 39: 83-89, 1995
- Borzelleca, J.F., Filer, L.J., Jr., Kinoshita, F.K., Gerrish, T.C., Kuo, P.K., and LaDu, B.N.: Evaluation of the safety of sodium pectate as a food ingredient. *Fd. Chem. Toxic.* 34:21-25, 1996
- Borzelleca, J.F.: A proposed model for safety assessment of macronutrient substitutes. *Reg. Tox. Pharm.* 23:S15-S18, 1996
- Steinberg, M., Borzelleca, J.F., et al: A new approach to the safety assessment of pharmaceutical excipients. *Reg. Tox. Pharm.* 24:149-154, 1996
- Berndt, W.O., Borzelleca, J.F., Flamm, W.G., and Munro, I.C.: Erythritol: A Review of Biological and Toxicological Studies. *Reg Tox. Pharm.* 24:S191-198, 1996
- Hallagan, J.B., LaDu, B.N., Pariza, M.W., Putnam, J.M., and Borzelleca, J.F.: Assessment of Cassia Gum. *Fd. Chem. Toxic.* 35:625-632, 1997

PUBLICATIONS

Graham, D.M., Pariza, M.W., Glaze, W.H., Newell, G.W., Erdman, J.W., and Borzelleca, J.F.: Use of Ozone in Food Processing. *Fd. Tech.* June 1997

Borzelleca, JF: Paracelsus: Herald of Modern Toxicology. *Toxicological Sciences* 53: 2-4. 1999

ABSTRACTS

Borzelleca, J.F. and Manthei, R.W.: Influence of dehydration on pentobarbital sleeping time in mice. *Fed. Proc.* 15: 403, 1956.

Borzelleca, J.F.: The effect of blood pH on barbiturate sleeping time in mice. *Fed. Proc.* 16: 284, 1957.

Borzelleca, J.F.: Drug absorption from the urinary bladder. *Fed. Proc.* 18: 370, 1959.

Borzelleca, J.F.: Nicotine absorption from the urinary bladder of the dog. *Fed. Proc.* 19: 391, 1960.

Borzelleca, J.F., Bowman, E.R. and McKennis, H., Jr.: Depressor effects arising from (-)-cotinine. *Pharmacologist* 2: 72, 1960.

Borzelleca, J.F.: Influence of saline infusions on the course of barbiturate intoxication. *Pharmacologist* 3: 63, 1961.

Borzelleca, J.F.: Drug absorption from the urinary tract of the rat. *Nicotine.Fed. Proc.* 21: 451, 1962.

Borzelleca, J.F.: Drug movement from the isolated urinary bladder of the rabbit. *Fed. Proc.* 22: 661, 1963.

Borzelleca, J.F.: Studies on the mechanisms of drug movement from the isolated urinary bladder. *Pharmacologist* 6: 178, 1964.

Kim, K.S., Borzelleca, J.F., McKennis, H., Jr. and Bowman, E.R.: Effects of cotinine and other nicotine metabolites *in vitro* on duodenum and ileum segments. *Fed. Proc.* 23: 330, 1964.

Borzelleca, J.F. and Doyle, H.: Salivary excretion of drugs. *Fed. Proc.* 24: 546, 1965.

Cherrick, H. and Borzelleca, J.F.: Salivary excretion of drugs. *Antibiotics. Toxicol. Appl. Pharmacol.* 7: 481, 1965.

Wooles, W.R. and Borzelleca, J.F.: Prolongation of barbiturate sleeping time in mice by stimulation of the RES. *J.R.E.S.* 1: 574, 1965.

Borzelleca, J.F.: Salivary excretion of glucose, salicylate, penicillin. *Fed. Proc.* 24: 564, 1966.

Lowenthal, W. and Borzelleca, J.F.: Rectal absorption of salicylates. *Toxicol. Appl. Pharmacol.* 8: 347, 1966.

Bernstein, S. and Borzelleca, J.F.: The effect of dimethylsulfoxide on drug transfer from the urinary bladder. *Va. J. Sci.* 18: 195, 1967.

Kim, K.S. and Borzelleca, J.F.: Pharmacological effects of some nicotine metabolites and related compounds. *Fed. Proc.* 26: 683, 1967.

Mullen, K. and Borzelleca, J.F.: Predictive model for blood glucose concentration in the dog. *Va. J. Sci.* 18: 200, 1967.

Schwartz, S.L. and Borzelleca, J.F.: Adrenergic blood pressure responses in the shark. *Proc. Shark Res. Panel of Am. Inst. Biol. Sci.*, 26 April 1968.

ABSTRACTS

- Schwartz, S.L. and Borzelleca, J.F.: Adrenergic responses in the shark. *Toxicol. Appl. Pharmacol.* 12: 307, 1968.
- Wynn, J.E., van't Riet, B. and Borzelleca, J.F.: Excretion and toxicity of EGTA and EDTA after oral administration to rats. *Fed. Proc.* 271: 465, 1968.
- van't Riet, B., O'Rear, C.E., Wynn, J.E. and Borzelleca, J.F.: Effect of EGTA and EDTA on bladder stone formation in rats. *Toxicol. Appl. Pharmacol.* 14: 638, 1969.
- Borzelleca, J.F. and van't Riet, B.: Hydrolysis and excretion of esters of EDTA and EGTA after oral administration to rats. *Va. J. Sci.* 29: 143, 1970.
- Borzelleca, J.F., Larson, P.S., Hennigar, G.R. and Kuchar, E.J.: A toxicological evaluation of pentachloronitrobenzene (PCNB). *Pharmacologist* 12: 208, 1970.
- Borzelleca, J.F.: The role of pharmacology in the training of toxicologists. *Pharmacologist* 12: 217, 1970.
- Putney, J.W., Jr. and Borzelleca, J.F.: A model for drug movement across the salivary epithelium. *Va. J. Sci.* 21: 147, 1970.
- Putney, J.W., Jr. and Borzelleca, J.F.: Factors modifying excretion of salicylate by the dog, comparison of urinary and salivary routes. *J. Toxicol. Appl. Pharmacol.* 16: 23, 1970.
- Putney, J.W., Jr. and Borzelleca, J.F.: Studies on salicylate biotransformation by the salivary gland. *Pharmacologist* 12: 272, 1970.
- Borzelleca, J.F., Larson, P.S., Hennigar, G.R. and Kuchar, E.J.: A toxicologic evaluation of 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole (terrazole). *Toxicol. Appl. Pharmacol.* 19: 79, 1971.
- Putney, J.W., Jr. and Borzelleca, J.F.: Mechanisms of ^{14}C -salicylate uptake by submaxillary gland slices. *Fed. Proc.* 30: 448, 1971.
- Putney, J.W., Jr. and Borzelleca, J.F.: Active uptake of ^{14}C -salicylic acid by rat kidney cortex slices. *Fed. Proc.* 31: 518, 1972.
- Putney, J.W., Jr. and Borzelleca, J.F.: Participation of extracellular hydrogen ion in the efflux of nicotine- ^{14}C from submaxillary gland cells. *Pharmacologist* 13: 518, 1972.
- Allen, M.A. and Borzelleca, J.F.: On the method of benzyl penicillin- ^{14}C potassium distribution in rat submaxillary gland. *Fed. Proc.* 32: 733, 1973.
- Allen, M.A. and Borzelleca, J.F.: On the method of diphenyl hydantoin distribution in rat submaxillary gland. *Pharmacologist* 15: 229, 1973.
- Jordan, R.L. and Borzelleca, J.F.: Teratogenic studies with pentachloronitrobenzene in rats. *Toxicol. Appl. Pharmacol.* 25: 454, 1973.
- Allen, M.A. and Borzelleca, J.F.: Diphenylhydantoin distribution in rat submaxillary gland: influence of age. *Fed. Proc.* 33: 525, 1974.
- Burnett, C.M., Agersborg, H.P.H., Jr., Borzelleca, J.F., Egle, Jr., E., Ebert, A.G., Pierce, E.C., Kirschman, J.C. and Scala, R.A.: Teratogenic studies with certified colors in rats and rabbits. *Toxicol. Appl. Pharmacol.* 29: 121, 1974.

ABSTRACTS

Pierce, E.G., Agersborg, H.P.K., Jr., Borzelleca, J.F., Burnett, C.M., Egle, E., Ebert, A.G., Kirschman, J.C. and Scala, R.A.: Multigeneration reproduction studies with certified colors in rats. *Toxicol. Appl. Pharmacol.* 29: 121, 1974.

Adams, M., Wedig, J.H., Jordan, R., Smith, L., Henderson, R. and Borzelleca, J.F.: Excretion and metabolism of three 2-6-¹⁴C Omadines following intravenous injection in female Yorkshire pigs. *Toxicol. Appl. Pharmacol.* 33: 180, 1975.

Egle, J.L., Jr., Borzelleca, J.F. and Long, J.E.: An evaluation of the cardiac sensitizing potential of Scotchgard brand fabric protector. *Toxicol. Appl. Pharmacol.* 33: 154, 1975.

Jordan, R.L. and Borzelleca, J.F.: Teratogenic studies with zinc Omadine in swine. *Anat. Rec.* 18: 388, 1975.

McConnell, W.R., Borzelleca, J.F. and Chambers, J.W.: The effects of delta-9-tetrahydrocannabinol (THC) on electrically stimulated saliva from cat submaxillary gland. *Fed. Proc.* 34: 782, 1975.

Smith, L.W., Borzelleca, J.F. and Bowman, E.R.: Application of isolated cell suspensions to the study of membrane phenomena in mammalian salivary cells. *Fed. Proc.* 34: 752, 1975.

Wrenn, J.M. and Borzelleca, J.F.: Effect of phenobarbital and pentobarbital on the transport of diphenylhydantoin in salivary tissues and saliva. *Fed. Proc.* 34: 573, 1975.

Egle, J.L., Jr., Gochberg, B.J. and Borzelleca, J.F.: The distribution of ¹⁴C-Kepon in the rat. *Pharmacologist* 18: 195, 1976.

McConnell, W.R. and Borzelleca, J.F.: On the method of 3H-delta-9 tetrahydrocannabinol (3H-delta-9-THC) distribution in the submaxillary gland of the rat. *Pharmacologist* 18: 149, 1976.

McConnell, W.R., Borzelleca, J.F. and Dewey, W.L.: The mechanism by which delta-9-tetrahydrocannabinol (THC) produces a decrease in salivary flow following electrical stimulation. *Fed. Proc.* 35: 644, 1976.

McCoy, W.D., Kuchar, E.J., Klein, H.H. and Borzelleca, J.F.: Biotransformation and distribution of pentachloronitrobenzene in chickens. *Toxicol. Appl. Pharmacol.* 37: 175, 1976.

Schumann, A.M. and Borzelleca, J.F.: The potential methemoglobin and Heinz body inducing capacity of pentachloronitrobenzene (PCNB) in the cat. *Toxicol. Appl. Pharmacol.* 37: 171, 1976.

Schumann, A.M., Bloom, A.S., Dewey, W.L., Harris, L.S. and Borzelleca, J.F.: Development of central catecholamine systems in the postnatal rat brain. *The Pharmacologist* 18: 243, 1976.

Smith, L.W. and Borzelleca, J.F.: Uptake of cadmium in rat submaxillary slices. *The Pharmacologist* 18: 196, 1976.

Bagshaw, B., Schumann, A., Borzelleca, J. and Dewey, W.: The effects of chloroform and bromoform on the noradrenergic and dopaminergic systems of the mouse brain. *Pharmacologist* 9: 200, 1977.

Barrett, B.A., Sanders, V.M., Borzelleca, J.F., Munson, E.: Growth rates and tumor takes in mice with transplanted tumors exposed to halomethanes. *Va. J. Sci.* 28: 100, 1977.

Brady, K.T., Sanders, V.M., Borzelleca, J.F. and Munson, A.E.: The acute toxicity of the halomethanes: drinking water contaminants. *Va. J. Sci.* 28: 100, 1977.

ABSTRACTS

- Martin, B.R., Dewey, W.L., Beckner, J.S. and Borzelleca, J.F.: Synthesis and metabolism of brain serotonin in mice following acute exposure to several haloalkanes. *Toxicol. Appl. Pharmacol.* 19: 200, 1977.
- Munson, A., Sanders, V., Borzelleca, J. and Barnes, D.: Toxicologic studies on adult and neonatal mice exposed to the trichloromethanes: drinking water contaminants. *Pharmacologist* 19: 200, 1977.
- Munson, A.E., Sanders, V.M., Barrett, B.A. and Borzelleca, J.F.: Functional activity of the reticuloendothelial system in mice exposed to haloalkanes for ninety days. *J. Reticuloendo. Soc.* 22: 17a, 1977.
- Sanders, V.M., Barrett, B.A., Borzelleca, J.F. and Munson, A.E.: Reticuloendothelial system activity and cell mediated immune responsiveness in mice exposed to polychlorinated biphenyls. *J. Reticuloendo. Soc.* 22: 16a, 1977.
- Schumann, A.M., Dewey, W.L. and Borzelleca, J.F.: The effects of triethyllead on central catecholamine function in the adult rat. *Toxicol. Appl. Pharmacol.* 41: 208, 1977.
- Schumann, A.M., Dewey, W.L., Borzelleca, J.F. and Alphin, R.S.: The effects of lead acetate on central catecholamine function in the postnatal mouse. *Fed. Proc.* 36: 405, 1977.
- Smith, L.W. and Borzelleca, J.F.: The excretion of cadmium and mercury in saliva. *Toxicol. Appl. Pharmacol.* 41: 153, 1977.
- Smith, L.W., Ismay, J.A. and Borzelleca, J.F.: Movement of mercury in rat submaxillary slices. *Fed. Proc.* 36: 355, 1977.
- Carmines, E.L., Burkhalter, J.A., Carchman, R.A. and Borzelleca, J.F.: Inhibitory effects of chloroform on P388D macrophage cell. *Fed. Proc.* 37: 320, 1978.
- Dougherty, R.W., Simon, G.S., Campbell, K.I. and Borzelleca, J.F.: Failure of 2,4-dinitrotoluene to induce dominant lethal mutations in the rat. *Pharmacologist* 20: 155, 1978.
- Larson, P.S., Hennigar, G.R., Lane, R.W. and Borzelleca, J.F.: Acute, subchronic and chronic toxicological studies with kepone. *Toxicol. Appl. Pharmacol.* 95: 331, 1978.
- Munson, A.E., Sanders, V.M., Borzelleca, J.F., Tardiff, R.G. and Barrett, B.A.: Reticuloendothelial system function in mice exposed to four haloalkane drinking water contaminants. *Toxicol. Appl. Pharmacol.* 45: 329, 1978.
- Schuller, G.B., Kauffmann, B.M., Borzelleca, J.F., Sanders, V.M. and Munson, A.E.: Effect of four haloalkanes on humoral and cell mediated immunity in mice. *Toxicol. Appl. Pharmacol.* 45: 329, 1978.
- Simon, G.S., Carchman, R.A. and Borzelleca, J.F.: Diabetes: responses to selected pharmacologic agents. *Pharmacologist* 20: 151, 1978.
- Simon, G.S., Kipps, B.R., Tardiff, R.G. and Borzelleca, J.F.: Failure of Kepone and hexachlorobenzene to induce dominant lethal mutations in the rat. *Toxicol. Appl. Pharmacol.* 45: 330, 1978.
- Smith, S.H., Sanders, V.M., Barrett, B.A., Borzelleca, J.F. and Munson, A.E.: Immunotoxicological evaluation on mice exposed to polychlorinated biphenyls. *Toxicol. Appl. Pharmacol.* 45: 330, 1978.
- Zimmerman, M.L., Lane, R.W., Skalsky, H.L. and Borzelleca, J.F.: Excretion of carbaryl into saliva and its effect on cholinesterase. *10th Inter-American Conf. on Toxicol. and Occupational Med.*, p. 47, 1978.

ABSTRACTS

- Zimmerman, M.L., May, R.G. and Borzelleca, J.F.: Excretion of carbaryl into the saliva of the rat. *Toxicol. Appl. Pharmacol.* 45: 35, 1978.
- Balster, R.L., Burkhalter, J. and Borzelleca, J.F.: Behavioral toxicity evaluation of four halomethane contaminants of drinking water in adult mice. *Fed. Proc.* 38: 846, 1979.
- Carmines, E.L., Carchman, R.A. and Borzelleca, J.F.: *in vitro* effects of Kepone. *Va. J. Sci.* 30: 89, 1979.
- Borzelleca, J.F., Skalsky, H.L. and Riddle, B.L.: Effects of dibromochloromethane in drinking water on reproduction and development in mice. *Fed. Proc.* 39: 999, 1980.
- Carmines, E.L., Carchman, R.A. and Borzelleca, J.F.: Analysis of the interactions between paraquat and DNA. *Fed. Proc.* 39: 545, 1980.
- Balster, R.L., Kallman, M.J. and Borzelleca, J.F.: Behavioral toxicity evaluation of trihalomethane contaminants of drinking water. *Health Effects of Drinking Water Symposium*, 1981.
- Carchman, R.A., Cardlin, E.L., Skalsky, H.L. and Borzelleca, J.F.: The effects of selected water disinfectant products on testicular DNA metabolism. *Health Effects of Drinking Water Symposium*, 1981.
- Kallman, M.J., Balster, R.L., Kaempf, G.L. and Borzelleca, J.F.: Behavioral toxicity evaluation of chloral in adult mice. *Fed. Proc.* 40: 698, 1981.
- Tarka, S.M., Jr., Keeney, P.G., and Borzelleca, J.F.: The effect of pretreatment with dietary cocoa on growth and reproductive performance in young and adult rats. *Fed. Proc.* 40: 668, 1981.
- Lane, R.W., Carchman, R.A. and Borzelleca, J.F.: Characterization of DNA metabolism in mouse primary spermatocytes. *Toxicologist* 1: 39(#143), 1981.
- Riddle, B.L., Carchman, R.A. and Borzelleca, J.F.: Effects of 1,2-dichloroethane and 1,1,1-trichloroethane in drinking water on reproduction and development in mice. *Toxicologist* 1: 26 (#95), 1981.
- Tarka, S.M., Jr., Keeney, P.G. and Borzelleca, J.F.: A comparison of the effects of methylxanthine-containing food stuffs on reproductive capacity in rats. *Toxicologist* 1: 147 (#533), 1981.
- Borzelleca, J.F., Hallagan, J., Reese, C., Goldenthal, E. and Hogan, G.: Chronic oral toxicity/carcinogenicity studies of food, drug and cosmetic colors in CD-1 mice. *J. Am. Coll. Tox.* 2: 240 (#108), 1982.
- Charles, J.L., Carchman, R.A., Kram, D. and Borzelleca, J.F.: Time course of *in vivo* induction of sister chromatid exchange by ethylnitrosourea and methylnitrosourea. *Toxicologist* 2: 175 (#613), 1982.
- Hayes, J.R., Condie, L.W., Jr., and Borzelleca, J.F.: Kinetics of naphthalene (NTL) covalent binding to hepatic DNA, RNA and protein in CD-1 mice. *J. Am. Coll. Tox.* 3: 144, 1982.
- Lane, R.W., Coles, R.B., Carchman, R.A. and Borzelleca, J.F.: Phorbol diester receptors on HL-60 human promyelocytic leukemia cells. *Toxicologist* 2: 105 (#373), 1982.
- Seyler, D., East, J. and Borzelleca, J.F.: Cadmium depression of mouse *in vitro* fertilization. *Toxicologist* 2: 238 (#764), 1982.
- Borzelleca, J.F., Hallagan, J., Reese, C., Goldenthal, E. and Hogan, G.: Chronic oral toxicity/carcinogenicity studies of food, drug and cosmetic colors in CD rats. *Toxicologist* 3: 129 (#514), 1983.
- Condie, L.W., Hayes, J.R. and Borzelleca, J.F.: Acute and subchronic oral toxicity of 2,4-dichlorophenol (2,4-DCP) in male and female CD-1 mice. *Pharmacologist* 25: 228, 1983.

ABSTRACTS

Hayes, J.R. and Borzelleca, J.F. Implications of nutrient-drug interactions. Proc. Ann Meeting Inst. Fd. Technol., 1983.

Hayes, J.R. and Borzelleca, J.F.: Diet-nutrient interactions. Proc. Ann. Mtg. of the Am. Diet. Assoc., 1983.

Hayes, J.R., Condie, L.W., Jr., and Borzelleca, J.F.: Pharmacokinetics of oral naphthalene (NTL) in CD-1 mice. *Toxicologist* 3: 161 (#644), 1983.

Kallman, M.J., Borzelleca, J.F. and Condie, L., Jr.: Behavioral toxicity of naphthalene in adult mice. *J. Am. Coll. Tox.* 2: 247 (#136), 1983.

Kessler, F.K., Charles, J.L., Borzelleca, J.F. and Carchman, R.A.: Effects of chlorinated phenols on mouse bone marrow sister chromatid exchange. *J. Am. Coll. Tox.* 2: 249 (#142), 1983.

Lane, R.W., Carchman, R.A. and Borzelleca, J.F.: Phorbol diester (PDE) binding and oxygen metabolism of differentiated HL-60 cells. *Toxicologist* 3: 144 (#575), 1983.

Shopp, G.M., White, K.L., Jr., Holsapple, M.P., Barnes, D.W., Condie, L.W., Jr. and Borzelleca, J.F.: General toxicology and immunotoxicology of mice exposed to naphthalene (NAP). *Toxicologist*, 3: 57 (#226), 1983.

Smith, B., Lane, R.W., Carchman, R.A. and Borzelleca, J.F.: A comparison of the reversibility of phorbol diester induced changes in macrophage morphology. *Toxicologist* 3: 144 (#574), 1983.

Borzelleca, J.F., Hayes, J.R. and Condie, L.: Toxicological evaluation of selected chlorinated phenols and haloacetonitriles. Proc. of 5th International Conf. on Water Chlorination: Environmental Impact and Health Effects 1: 100, 1984.

Hayes, J.R., Condie, L. and Borzelleca, J.F.: Subchronic toxicity of carbon tetrachloride administered by oral gavage to CD-1 mice. *Toxicologist* 4: 183 (#730), 1984.

Hayes, J.R., Condie, L.W. and Borzelleca, J.F.: Acute and 14-day continuous dosing toxicity of dichloroacetonitrile (DCA) and dibromoacetonitrile (DBA). *Pharmacologist* 26: 233, 1984.

Condie, L.W. Hayes, J.R. and Borzelleca, J.F.: Acute, 14-day and subchronic toxicity of potassium picloram (PIC) administered to rats via the drinking water. *Toxicologist* 5: 222, 1985

Capen, C.C., Nishikawa, S., Ingbar, S.H., Braverman, L.E., and Borzelleca, J.F.: Mechanisms of thyroid oncogenesis by chronic erythrosine (red. no. 3) feeding: ultrastructural and morphometric evaluation of thyroid glands and changes in circulating levels of thyroid hormones and thyrotropin (TSH). Abstract No. 48, 75th Annual Meeting of the International Academy of Pathology, New Orleans, 10-14 March, and published in *Laboratory Investigations* 54: 54 a, 1986.

Lamb, R.G., Bush, S.R., Condie, L.W., and Borzelleca, J.F.: Influence of chlorinated hydrocarbon mixtures on cultured hepatocyte function. *Pharmacologist* 28: 180, 1986.

Lamb, R.G., Coleman, J.B., Condie, L.W., and Borzelleca, J.F.: Influence of chlorinated hydrocarbons on cultured hepatocyte function. *Toxicologist* 6: 116 (#470), 1986.

Granger, R.H., Coleman, J.B., Condie, L.W., Lamb, R.G. and Borzelleca, J.F.: Effect of vehicle on the relative uptake of haloalkanes administered by gavage. *Toxicologist* 7: 265 (#1060), 1987.

Lamb, R.G., Coleman, J.B., Granger, H., Condie, L.W. and Borzelleca, J.F.: The influence of chlorinated hydrocarbons on hepatocyte function *in vivo* and *in vitro*. *Toxicologist* 7: 267 (#1068), 1987.

Coleman, J.B., Condie, L.W., Borzelleca, J.F. and Lamb, R.G.: The influence of structural analogues of carbon tetrachloride (CCl₄) on hepatocyte functions *in vitro*. *Toxicologist* 8: 96 (#381), 1988.

ABSTRACTS

Granger, R.H., O'Hara, T.M., Condie, L.W., and Borzelleca, J.F.: A study of the joint action of carbon tetrachloride (CCl₄) and trichloroethylene (C₂HCl₃) following simultaneous gavage administration in the rat. *Toxicologist* 8: 95 (#378), 1988.

O'Hara, T.M., Granger, R.H., Condie, L.W. and Borzelleca, J.F.: A study of the joint hepatotoxic action of carbon tetrachloride (CCl₄) and chloroform (CHCl₃) following simultaneous gavage administration in the rat. *Toxicologist* 8: 96 (#380), 1988.

Borzelleca, J.F., O'Hara, T.M., Gennings, C. and Condie, L.W., A CCl₄-CHCl₃ interaction study in isolated hepatocytes-the role of P-450 metabolism. *Toxicologist* 9: 58 (#229), 1989.

Lamb, R.G., Gennings, C., Borzelleca, J.F., and Condie, L.W.: Toxic Interactions between carbon tetrachloride (CCl₄) and chloroform (CHCl₃). *Toxicologist* 9: 59 (#233), 1989.

O'Hara, T.M., Borzelleca, J.F. and Condie, L.W.: A CCl₄/CHCl₃ interaction study in isolated hepatocytes-selection of a vehicle. *Toxicologist* 9: 59 (#235), 1989.

Borzelleca, J.F., Gennings, C., Bercz, P. and Lamb, R.G.: Toxic interactions between carbon tetrachloride (CCl₄) and perchloroethylene (PCE) in cultured rat hepatocytes. *Toxicologist* 10: 54 (#213), 1990.

Lamb, R.G., Gennings, C., Borzelleca, J.F. and Bercz, P.: Toxic interactions between carbon tetrachloride (CCl₄) and trichloroethylene (TCE) in cultured rat hepatocytes. *Toxicologist* 10: 53 (#212), 1990.

Wolfe, G., Myers, B., Lemen, J., Lauer, W., Johns, F., Condie, L. and Borzelleca, J.: Preliminary report of the findings of the health effects for Denver's potable reuse demonstration project. *Toxicologist* 10: 176 (#704), 1990.

Egle, J.L., Jr., Borzelleca, J.F. and Harris, L.S.: Acute and subchronic toxicity of Levo-alpha- acetyl-methadol (LAAM) and Levo-alpha-acetyl-normethadol (NORLAAM) in male and female rats. *Toxicologist* 11: 149 (#521), 1991

Weiner, M.L., Steinberg, M., Borzelleca, J.F., Enters, E.K., Hager, D.F., Kinoshita, F.K., Loper, A., Mitchell, D.B. and Tamulinas, C.B.: Proposed safety evaluation guidelines for new excipients. *Toxicologist* 13: 213 (#796), 1994

Borzelleca, J.F.: The safety evaluation of macronutrient substitutes. IFT Annual Meeting Abstracts #15-2, 1994

Borzelleca, J.F.: Fat replacers. ACS meeting, 1995

Rice, R.G., Graham, D.M., Glaze, W.H., Pariza, M.W., Newell, G.W., Erdman, J.W., and Borzelleca, J.F.: Ozone preservation of Foods and Foodstuffs. 13th Ozone World Congress, October 1997, Kyoto, Japan

Lien, E., Boyle, F., Perry, Thompson, C., Borzelleca, J.F., and Wrenn, J.: Comparison of AIN-76A and AIN-93G Diets in Rats; a 13 Week Study. *Fed. Proc.*, 1998

Munro, E.C., Berndt, W.O., Borzelleca, J.F., Flamm, G., Lynch, B.S., Kennepohl, E., Bar, A. and Modderman, J.: Erythritol: An Interpretive Summary of Biochemical, Metabolic, Toxicological and Clinical Data. *Toxicologist* 38: , 1999

BOOKS and BOOK CHAPTERS

Skalsky, H.L., Lane, R.W. and Borzelleca, J.F.: "Excretion of carbaryl into saliva of the rat and its effect on cholinesterase". In: *Toxicology and Occupational Medicine* (W.B. Deichman, ed.), p. 349, 1979.

Borzelleca, J.F. and Carmines, E.L.: "New drug evaluation: safety assessment". In: *Program for Applied Research on Fertility Regulation*, 1980.

000342

JFB/CV/05/2000

BOOKS and BOOK CHAPTERS

Hayes, J.F. and Borzelleca, J.F.: "Biodisposition of environmental chemicals by animals". In: *Animal Products in Human Nutrition* (D. Beitz and R. Hansen, eds.), Chap. 11, p. 225. Academic Press, New York, 1982.

Borzelleca, J.F.: "Neurobehavior toxicological testing". *Pharmacodependence and neurobehavioral toxicology. Quo Vadis ?*, Symposium "Quo Vadis ?", Sanofi Group, Montpellier, France, p. 115, 1983.

Schwartz, S.L. and Borzelleca, J.F.: "Toxicology of polyvinylpyrrolidone". *Proceedings of the International Symposium on Povidone* (G.A. Digenis, Ed.), College of Pharmacy, University of Kentucky, Lexington, KY, p. 234, 1983.

Borzelleca, J.F., Hallagan, J. and Reese, C.: "Food, Drug and Cosmetic Colors: Toxicological Considerations." *ACS Symposium Series, No. 234, Xenobiotics in Foods and Feeds.* (Finley, J.W. and Schwass, D.E., eds.), Chap. 20, p.311. ACS, Washington, D.C., 1983

Borzelleca, J.F.: "Extrapolation of animal data to man". In: *Toxicology Laboratory Design and Management for the 80's and Beyond* (Tegeris, A.S., Ed); Vol. 1 of *Concepts in Toxicology*, Homburger, F., Series Ed.), 1984.

Borzelleca, J.F.: "Current concepts in reproductive toxicology". In: *Clinics in Laboratory Medicine, Symposium on Environmental and Occupational Health Hazards*, Vol. 4 (R.V. Blanke, ed.), W.B. Saunders Co., Philadelphia, 1984.

Borzelleca, J.F., Condie, L.W., and Hayes, J.R.: "Toxicological evaluation of selected chlorinated phenols". In *Water Chlorination, Chemistry, Environmental Impact and Health Effects.* (R.L. Jolley, R.J. Bull, W.P. Davis, S. Katz, M.H. Roberts, Jr., V.A. Jacobs). Volume 5, Chap. 26, p.331. Lewis Publishers, Inc., Ann Arbor, Michigan, 1985.

Robinson, B.V., Sullivan, F.M., Borzelleca, J.F. and Schwartz, S.L.: *PVP: A Critical Review of the Kinetics and Toxicology of Polyvinylpyrrolidone (Povidone).* Lewis Publishers, Inc., Ann Arbor, Michigan. 1990

Borzelleca, J.F. and Hallagan, J.B.: "Safety and Regulatory Status of Food, Drug, and Cosmetic Colors." *ACS Symposium Series, No. 484, Food Safety Assessment.* (Finley, J.W., Robinson, S.F., and Armstrong, D.J., eds.), Chap. 31, p.377. ACS, Washington, DC. 1992

Borzelleca, J.F.: "Foods of the Future: What Will We Be Eating in the Next Century?" In *Practical Handbook of Nutrition in Clinical Practice* (Kirby, D.F. and Dudrick, S.J., eds.), Chap. 16, p.279. CRC Press, Inc., Boca Raton, Fl. 1994

Borzelleca, J.F.: "History of Toxicology." In *Principles and Methods of Toxicology* (Hayes, A.W., editor), edition 3, Chap. 1, p.1., Raven Press, New York, NY. 1994

Matt, D.W. and Borzelleca, J.F.: "Toxic Effects on the Female Reproductive System During Pregnancy, Parturition, and Lactation." In *Reproductive Toxicology* (Witorsch, R.J., editor), edition 2, chapter 10, p. 175. Raven Press, New York, NY. 1995

Borzelleca, J.F.: "Food-Borne Health Risks: Food Additives, Pesticides and Microbes." In *Nutrition Policy in Public Health* (Bronner, F., editor). Chap. 3, p.33, Springer Publishing Co. New York, NY. 1997

Rice, R.G., Graham, D.M., Glaze, W.H., Pariza, M.W., Newell, G.W., Erdman, J.W., and Borzelleca, J.F.: *Ozone Preservation of Foods and Foodstuffs.* 13th Ozone World Congress, Kyoto, Japan, October 1997

Borzelleca, J.F. and Weiner, M.L. : "Development of Safety Evaluation Guidelines." In *Excipient Toxicity and Safety* (Weiner, M. L. and Kotkoskie, L. A., editors). Chapter 5, p.101. Marcel Dekker, Inc., New York, N.Y. 1999

**Contributing authorship on the following publications of the Life Sciences Research Office,
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Research Office, Federation of American Societies of Experimental Biology (FASEB):

- Evaluation of the health aspects of iron and iron salts as food ingredients. 1973.
- Evaluation of the health aspects of butylated hydroxytoluene as a food ingredient. 1973.
- Evaluation of the health aspects of certain zinc salts as food ingredients. 1973.
- Evaluation of the health aspect of pulps as they may migrate to food from packaging materials. 1973.
- Evaluation of the health aspects of propylene glycol and propylene glycol monostearate as food ingredients. 1973.
- Evaluation of the health aspects of alginates as food ingredients. 1973.
- Evaluation of the health aspects of agar-agar as a food ingredient. 1973.
- Evaluation of the health aspects of certain red and brown algae as food ingredients. 1973.
- Evaluation of the health aspects of cellulose and certain cellulose derivatives of food ingredients. 1973.
- Iodine in foods: chemical methodology and sources of iodine in the human diet. 1974.
- Evaluation of the health aspects of aconitic acid as a food ingredient. 1974.
- Evaluation of the health aspects of stannous chloride as a food ingredient. 1974.
- Evaluation of the health aspects of licorice, glycyrrhiza and ammoniated glycyrrhizin as food ingredients. 1974.
- Evaluation of the health aspects of caprylic acid as a food ingredient. 1974.
- Evaluation of the health aspects of sorbose as a food ingredient. 1974.
- Evaluation of the health aspects of sulfuric acid and sulfates as food ingredients. 1974.
- Evaluation of the health aspects of potassium iodide, potassium iodate, and calcium iodate as food ingredients. 1975.
- Evaluation of the health aspects of dextran as food ingredients. 1975.
- Evaluation of the health aspects of calcium oxide and calcium hydroxide as food ingredients. 1975.
- Evaluation of the health aspects of succinic acid as a food ingredient. 1975.
- Evaluation of the health aspects of certain calcium salts as food ingredients. 1975.
- Evaluation of the health aspects of glycerin and glycerides as food ingredients 1975
- Evaluation of the health aspects of dextrin and corn dextrin as food ingredients. 1975.
- Evaluation of the health aspects of sodium thiosulfate as a food ingredient. 1975.
- Evaluation of the health aspects of gelatin as a food ingredient. 1975.
- Evaluation of the health aspects of bile salts and ox bile extract as food ingredients. 1975.
- Evaluation of the health aspects of choline chloride and choline bitartrate as food ingredients. 1975.

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- Evaluation of the health aspects of aluminum compounds as food ingredients. 1975.
- Evaluation of the health aspects of tallow, hydrogenated tallow, stearic acid, and calcium stearate as food ingredients. 1975.
- Evaluation of the health aspects of phosphates as food ingredients. 1975.
- Evaluation of the health aspects of the tocopherols and a-tocopheryl acetate as food ingredients. 1975.
- Evaluation of the health aspects of sorbic acid and its salts as food ingredients. 1975.
- Evaluation of the health aspects of hydrogenated fish oil as a food ingredient. 1975.
- Evaluation of the health aspects of beeswax (yellow or white) as a food ingredient. 1975.
- Evaluation of the health aspects of inositol as a food ingredient. 1975.
- Evaluation of the health aspects of malic acid as a food ingredient. 1975.
- Evaluation of the health aspects of Japan Wax as a substance migrating to food from cotton or cotton fabrics used in dry food packaging. 1976.
- Evaluation of the health aspects of carnauba wax as a food ingredient. 1976.
- Evaluation of the health aspects of sulfamic acid as it may migrate to foods from packaging materials. 1976.
- Evaluation of the health aspects of hydrosulfites as they may migrate to foods from packaging materials. 1976.
- Evaluation of the health aspects of gum guaiac as a food ingredient. 1976.
- Evaluation of the health aspects of tall oil as it may migrate to foods from packaging materials. 1976.
- Evaluation of the health aspects of corn sugar (dextrose), corn syrup and invert sugar as food ingredients. 1976.
- Evaluation of the health aspects of sucrose as a food ingredient. 1976.
- Evaluation of the health aspects of sulfiting agents as food ingredients. 1976.
- Evaluation of the health aspects of glycerophosphates as food ingredients. 1976.
- Evaluation of the health aspects of magnesium salts as food ingredients. 1976.
- Evaluation of the health aspects of sodium hydroxide and potassium hydroxide as food ingredients. 1976.
- Evaluation of the health aspects of adipic acid as a food ingredient. 1976.
- Evaluation of the health aspects of hydrogenated soybean oil as a food ingredient.
- Evaluation of the health aspects of formic acid, sodium formate, and ethyl formate as food ingredients. 1976.
- Evaluation of the health aspects of lard and lard oil as they may migrate to foods from packaging materials. 1976.
- Evaluation of the health aspects of pyridoxine and pyridoxine hydrochloride as food ingredients. 1977.

**Contributing authorship on the following publications of the Life Sciences Research Office,
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- Evaluation of the health aspects of papain as a food ingredient. 1977.
- Evaluation of the health aspects of hypophosphites as food ingredients. 1977.
- Evaluation of the health aspects of coconut oil, peanut oil, and oleic acid as they migrate to food from packaging materials, and linoleic acid as a food ingredient. 1977.
- Evaluation of the health aspects of pectin and pectinates as food ingredients. 1977.
- Evaluation of the health aspects of tannic acid as a food ingredient. 1977.
- Evaluation of the health aspects of rennet as a food ingredient. 1977.
- Evaluation of the health aspects of acetic acid and sodium acetate as food ingredients. 1977.
- Evaluation of the health aspects of sodium oleate and sodium palmitate as substances migrating to food from paper and paperboard used in food packaging. 1977.
- Evaluation of the health aspects of corn silk as a food ingredient. 1977.
- Evaluation of the health aspects of bentonite and clay (kaolin) as food ingredients. 1977.
- Evaluation of the health aspects of citric acid, sodium citrate, potassium citrate, calcium citrate, ammonium citrate, triethyl citrate, isopropyl citrate, and stearyl citrate as food ingredients. 1977.
- Evaluation of the health aspects of lactic acid and calcium lactate as food ingredients. 1978.
- Evaluation of the health aspects of calcium pantothenate, sodium pantothenate, and D-pantothenyl alcohol as food ingredients. 1978.
- Evaluation of the health aspects of Vitamin B12 as a food ingredient. 1978.
- Evaluation of the health aspects of Vitamin D2 and Vitamin D3 as food ingredients. 1978.
- Evaluation of the health aspects of caffeine as a food ingredient. 1978.
- Evaluation of the health aspects of certain glutamates as food ingredients. 1978.
- Evaluation of the health aspects of protein hydrolyzates as food ingredients. 1978.
- Evaluation of the health aspects of butylated hydroxyanisole as a food ingredient. 1978.
- Evaluation of the health aspects of sodium, potassium, magnesium and zinc gluconates as food ingredients. 1978.
- Evaluation of the health aspects of urea as a food ingredient. 1978.
- Evaluation of the health aspects of thiamin hydrochloride and thiamin mononitrate as food ingredients. 1978.
- Evaluation of the health aspects of biotin as a food ingredient. 1978.
- Evaluation of the health aspects of ascorbic acid, sodium ascorbate, calcium ascorbate, erythorbic acid, sodium erythorbate, and ascorbyl palmitate as food ingredients. 1979.
- Evaluation of the health aspects of propionic acid, calcium propionate, sodium propionate, dilauryl thiodipropionate, and thiodipropionic acid as food ingredients. 1979.

**Contributing authorship on the following publications of the Life Sciences Research Office,
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Evaluation of the health aspects of casein, sodium caseinate, and calcium caseinate as food ingredients. 1979.

Evaluation of the health aspects of nickel as a food ingredient. 1979

Evaluation of the health aspects of soy protein isolates as food ingredients. 1979.

Evaluation of the health aspects of carotene (B-carotene) as a food ingredient. 1979.

Evaluation of the health aspects of nitrogen, helium, propane, n-butane, isobutane, and nitrous oxide as gases used in foods. 1979.

Evaluation of the health aspects of hydrogen peroxide as a food ingredient. 1979.

Evaluation of the health aspects of riboflavin and riboflavin-5-1-phosphate as food ingredients. 1979.

Evaluation of the health aspects of starch and modified starches as food ingredients. 1979.

Evaluation of the health aspects of carbon dioxide as a food ingredient. 1979.

Evaluation of the health aspects of sodium chloride and potassium chloride as food ingredients. 1979.

Evaluation of the health aspects of certain silicates as food ingredients. 1979.

Evaluation of the health aspects of manganous salts as food ingredients. 1979.

Evaluation of the health aspects of copper gluconate, copper sulfate, and cuprous iodide as food ingredients. 1979.

Evaluation of the health aspects of hydrochloric acid as a food ingredient. 1979.

Evaluation of the health aspects of lecithin as a food ingredient. 1979.

Evaluation of the health aspects of potassium acid tartrate, sodium potassium tartrate, sodium tartrate and tartaric acid as food ingredients. 1979.

Evaluation of the health aspects of starter distillate and diacetyl as food ingredients. 1980.

Vitamin A, Vitamin A Acetate, and Vitamin A Palmitate as food ingredients. 1980.

Evaluation of the health aspects of iron and iron salts as food ingredients. 1980.

Evaluation of the health aspects of protein hydrolyzates as food ingredients. 1980.

Evaluation of the health aspects of collagen as a food ingredient. 1981.

Evaluation of the health aspects of methyl polysilicones as food ingredients. 1981.

Evaluation of the health aspects of soya fatty acid amines as food ingredients. 1981.

Evaluation of the health aspects of activated carbon (charcoal) as a food processing aid. 1981.

Evaluation of the health aspects of smoke flavoring solutions and smoked yeast flavoring as food ingredients. 1981.

Evaluation of the health aspects of commint oil as a food ingredient. 1981.

Evaluation of the health aspects of a mixture. Evaluation of the health aspects of diferrous, dipotassium ferrous, and potassium ferrocyanides as finding agents in wine production. 1981.

**Contributing authorship on the following publications of the Life Sciences Research Office,
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Evaluation of the health aspects of wheat gluten, corn gluten, and zein as food ingredients. 1981.

Evaluation of the health aspects of peptones as food ingredients. 1981.

Evaluation of the health aspects of shellac and shellac wax as food ingredients. 1981.

Evaluation of the health aspects of sodium metasilicate and sodium zinc metasilicate as food ingredients. 1981.

Evaluation of the health aspects of oat gum, okra gum, quince seed gum, and psyllium seed husk gum as food ingredients. 1982.

Contributing Authorship on the Following Publications of the National Academy of Sciences

Principles and Procedures for Evaluating the Toxicity of Household Substances.
Committee for the Revision of NAS Publication 1138, Committee on Toxicology, Assembly of Life Sciences,
National Research Council, National Academy of Sciences.
National Academy Press, Washington, D.C. 1977

Drinking Water and Health.
Safe Drinking Water Committee, Board on Toxicology and Environmental Health Hazards, Assembly of Life
Sciences, National Research Council, National Academy of Sciences
Volume 1, 1977; Volume 2, 1980, Volume 3, 1980
National Academy Press, Washington, D.C.

Estimating Consumer Exposure to Food Additives and Monitoring Trends in Use.
Food Additives Survey Committee, Food and Nutrition Board, Institute of Medicine, National Academy of
Sciences
National Academy Press, Washington, D.C. 1992

Examination of Dietary Recommendations for Salt-Cured, Smoked, and Nitrite-Preserved Foods
Pariza, M.W., Borzelleca, J.F., Cassens, R.G., Filer, L.J., and Kritchevsky, D.,
CAST Issue Paper Number 8, November 1997

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Instructor of Chemistry (Part-time), Northwestern University, Evanston, Illinois, 1966

Assistant Professor, University of Maryland, 1967-71

Associate Professor, University of Maryland, 1971-79

Visiting Scholar in Residence, University of Virginia, 1975-76

Professor of Chemistry, University of Maryland, 1979-present

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Chair, Department of Chemistry and Biochemistry, 1993-1998

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III. Academic Activities

000350

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000351

IV. Research, Teaching and Service

A. Publications (1970 - present; 12 publications prior to 1970)

13. B. B. Jarvis and R. O. Fitch, "Trapping of the Radical Intermediate in the Photochemical Rearrangement of an Allyl Chloride," *Chem. Commun.*, 408-409 (1970).
14. B. B. Jarvis and J. B. Young, III, "Diels-Alder Reaction of Tetrachloroethylene with Anthracene," *J. Org. Chem.*, **35**, 2088-2090 (1970).
15. B. B. Jarvis, "Free Radical Additions of Dibenzotricyclo[3.3.0.0.2,8]-3,6-octadiene," *J. Org. Chem.*, **35**, 924-927 (1970).
16. B. B. Jarvis, J. P. Govoni, and P. J. Zell, "Stereoselective Free-Radical Rearrangements," *J. Am. Chem. Soc.*, **93**, 913-918 (1971).
17. B. B. Jarvis, J. B. Young, III, and T. H. Yang, "Stereochemistry of the Reduction of Homobenzyl Halides," *J. Org. Chem.*, **37**, 797-800 (1972).
18. B. B. Jarvis, S. D. Dutkey, and H. L. Ammon, "Stereochemistry of 1,3-Eliminations from Dibromo Sulfoxides," *J. Am. Chem. Soc.*, **94**, 2136-2138 (1972).
19. B. B. Jarvis and J. P. Govoni, "Distal Effects in E2 Eliminations," *J. Org. Chem.*, **37**, 1568-1575 (1972).
20. B. B. Jarvis and J. C. Saukaitis, "Nucleophilic Displacements on Halogen Atom I. Reactivity of α -Halobenzyl Phenyl Sulfones Toward Triphenylphosphine," *Tetrahedron Letters*, 709-712 (1973).
21. F. G. Bordwell and B. B. Jarvis, "Stereochemistry of 1,3-Elimination Reactions," *J. Am. Chem. Soc.*, **95**, 3585-3592 (1973).
22. B. B. Jarvis and J. C. Saukaitis, "Nucleophilic Displacements on Halogen Atoms II. Kinetic Study of the Reactions of α -Halo Sulfones with Triphenylphosphines," *J. Am. Chem. Soc.*, **95**, 7708-7715 (1973).
23. B. B. Jarvis and M. M. Evans, "Nucleophilic Displacements on Halogen Atoms III. Reduction of α,α -Dichlorobenzyl Sulfoxides," *J. Org. Chem.*, **39**, 643-647 (1974).
24. J. F. Liebman and B. B. Jarvis, "Nucleophilic Displacements Reactions on Fluorine: General Phenomenon and Lone Pair Nucleophiles," *J. Fluorine Chem.*, **5**, 41-44 (1975).
25. B. B. Jarvis and W. P. Tong, "Synthesis of a New Class of Vinyl Betaines," *Synthesis*, 102-104 (1975).
26. B. B. Jarvis and H. E. Fried, "Chlorination of Disulfoxides," *J. Org. Chem.*, **40**, 1278-1280 (1975).

27. B. B. Jarvis and B. A. Marien, "Nucleophilic Displacements Reactions on Halogen Atoms V. Reactions of α -Halo Sulfones With Sterically Hindered Nucleophiles," *J. Org. Chem.*, **40**, 2587-2590 (1975).
28. B. B. Jarvis, R. L. Harper, Jr., and W. P. Tong, "Nucleophilic Displacements on Halogen Atoms VI. The Determination of α -values for the Carboxyl, Carboethoxy and Methylsulfonyl Groups," *J. Org. Chem.*, **40**, 3778-3780 (1975).
29. B. B. Jarvis, W. P. Tong, and H. L. Ammon, "Reactions of 2,3-Diphenylthiirene 1,1-Dioxide with Nucleophiles," *J. Org. Chem.*, **40**, 3189-3195 (1975).
30. B. B. Jarvis and W. P. Tong, "Nucleophilic Displacements on Halogen Atoms VII. Reactions of α -Halo Sulfones with Sodium Arenesulfonates," *J. Org. Chem.*, **41**, 1557-1560 (1976).
31. B. B. Jarvis and B. A. Marien, "Nucleophilic Displacements on Halogen Atoms VIII. Reactions of α -Halo Sulfones with Triarylphosphines, Alkyldiphenylphosphines, and Phosphites," *J. Org. Chem.*, **41**, 2182-2187 (1976).
32. S. M. Kupchan, B. B. Jarvis, R. G. Dailey, Jr., W. Bright, R. F. Bryan, and Y. Shizuri, "Baccharin, A Novel Potent Antileukemic Trichothecenes Triepoxide from *Baccharis megapotamica*," *J. Am. Chem. Soc.*, **98**, 7092-7093 (1976).
33. B. B. Jarvis and B. A. Marien, "Kinetic Study of the Reactions of Triarylphosphines with α -Halosulfones," *Phosphorus and Sulfur*, **1**, 177-178 (1976).
34. B. B. Jarvis and B. A. Marien, "Nucleophilic Displacements on Halogen Atoms IX. Reactions of Triarylphosphines with Halomethylpyridyl Phenyl Sulfones," *J. Org. Chem.*, **42**, 2676-2680 (1977).
35. S. M. Kupchan, D. R. Streelman, B. B. Jarvis, R. G. Dailey, Jr., and A. T. Sneden, "The Isolation of Potent New Antileukemic Trichothecenes from *Baccharis megapotamica*," *J. Org. Chem.*, **42**, 4221-4225 (1977).
36. B. B. Jarvis, G. P. Stahly, and H. L. Ammon, "A Novel New Heterocycle from the Reaction of Azide Ion with 2,3-Diphenylthiirene 1,1-Dioxide," *Tetrahedron Letters*, 3781-3784 (1978).
37. B. B. Jarvis, G. P. Stahly, and C. R. Curtis, "Antileukemic Activity of Fungal Metabolites: Verrucarin β -9,10-Epoxides," *Cancer Treat. Rep.*, **62**, 1585-1586 (1978).
38. B. B. Jarvis and P. E. Nicholas, "Base Decompositions of Azides Leading to Nitriles," *J. Org. Chem.*, **44**, 2951-2952 (1979).
39. B. B. Jarvis, J. O. Midiwo, G. P. Stahly, G. Pavanadasivam, and E. P. Mazzola, "Trichodermadiene: A New Trichothecene," *Tetrahedron Letters*, 787-788 (1980).

40. R. C. Kalbeck, G. W. Gokel, D. M. Dishong, C. J. Diamond, L. B. Hendry, B. B. Jarvis, W. A. Spein and E. D. Bransome, "Crown Ethers as Calcium Ionophores," *Dev. Biochem.*, **14**, 471 (1980).
41. B. B. Jarvis, G. P. Stahly, G. Pavanasasivam, and E. P. Mazzola, "Structure of Roridin J, A New Macrocyclic Trichothecene from *Myrothecium verrucaria*," *J. Antibiotics*, **33**, 256-258 (1980).
42. B. B. Jarvis and G. P. Stahly, "Reactions of 2,3-Diphenylthiirene 1,1-Dioxide with Nucleophiles," *J. Org. Chem.*, **45**, 2604-2609 (1980).
43. B. B. Jarvis and P. E. Nicholas, "Reactions of α -Azido Sulfones with Bases," *J. Org. Chem.*, **45**, 2265-2268 (1980).
44. B. B. Jarvis, G. P. Stahly, G. Pavanasasivam, and E. P. Mazzola, "Antileukemic Compounds Derived from the Chemical Modification of Macrocyclic Trichothecenes 1. Derivatives of Verrucaric Acid," *J. Med. Chem.*, **23**, 1054-1058 (1980).
45. G. P. Stahly, H. L. Ammon, and B. B. Jarvis, "The Structure of 2,6-Diphenyl-4-(E-1,2-diphenylinyl)-1,3,4,5-thiatriazine-1,1-Dioxide," *Acta Cryst.* **B36**, 2159-2163 (1980).
46. B. B. Jarvis, G. Pavanasasivam, C. E. Holmlund, T. DeSilva, G. P. Stahly, and E. P. Mazzola, "Biosynthetic Intermediates to the Macrocyclic Trichothecenes," *J. Am. Chem. Soc.*, **103**, 472-474 (1981).
47. B. B. Jarvis, J. O. Midiwo, T. DeSilva, and E. P. Mazzola, "Verrucaric Acid, A New Macrocyclic Trichothecene," *J. Antibiotics*, **34**, 120-121 (1981).
48. B. B. Jarvis, P. E. Nicholas, and J. O. Midiwo, "Thermolysis of α -Azido Sulfides, Sulfoxides, and Sulfones: Dependence of Mechanism on Oxidation State of Sulfur," *J. Am. Chem. Soc.*, **103**, 3878-3882 (1981).
49. B. B. Jarvis, J. O. Midiwo, D. Tuthill, and G. A. Bean, "Interaction Between the Antibiotic Trichothecenes and the Higher Plant *Baccharis megapotamica*," *Science*, **214**, 460-462 (1981).
50. B. B. Jarvis, G. P. Stahly, G. Pavanasasivam, J. O. Midiwo, T. DeSilva, C. E. Holmlund, E. P. Mazzola, and R. F. Geoghegan, Jr., "Isolation and Characterization of the Trichoverroids and New Roridins and Verrucarins," *J. Org. Chem.*, **47**, 1117-1124 (1982).
51. B. B. Jarvis, J. O. Midiwo, J. L. Flippen-Anderson, and E. P. Mazzola, "Stereochemistry of the Roridins," *J. Nat. Prod.*, **45**, 440-448 (1982).
52. R. Esmond, B. Fraser-Reid, and B. B. Jarvis, "Synthesis of Trichoverrin B and Its Conversion to Verrucaric Acid," *J. Org. Chem.*, **47**, 3358-3360 (1982).

53. B. B. Jarvis and E. P. Mazzola, "Macrocyclic and Other Novel Trichothecenes," *Acc. Chem. Res.*, **15**, 388-395 (1982).
54. B. B. Jarvis and C. B. Anderson, "Bisallyl and Bismethallyl Derivatives of 2,3-Dihydropyridine," *J. Heterocyclic Chem.*, **20**, 471-473 (1983).
55. B. B. Jarvis, R. M. Epply, and E. P. Mazzola, "Chemistry and Bioproduction of Macrocyclic Trichothecenes," In Trichothecenes-Chemical, Biological and Toxicological Aspects, Y. Ueno, (Ed.), Kodarsha Scientific, Tokyo, 1983, p. 20-38.
56. B. B. Jarvis, V. M. Vrudhula, "New Trichoverroids from *Myrothecium verrucaria*: 16-Hydroxytrichodermedienediols," *J. Antibiotics*, **36**, 459-461 (1983).
57. B. B. Jarvis, V. M. Vrudhula, J. O. Midiwo, and E. P. Mazzola, "New Trichothecenes from *Myrothecium verrucaria*: Verrol and 12,13-Deoxytrichodermediene," *J. Org. Chem.*, **48**, 2576-2580 (1983).
58. G. Pavanadasivam and B. B. Jarvis, "Microbial Transformation of Macrocyclic Trichothecenes," *Appl. Environ. Microbiol.*, **46**, 480-483 (1983).
59. B. B. Jarvis, V. M. Vrudhula, and G. Pavanadasivam, "Trichoverritone and 16-Hydroxyroridin L-2, New Trichothecenes from *Myrothecium roridum*," *Tetrahedron Letters*, **24**, 3539-3542 (1983).
60. B. B. Jarvis, J. O. Midiwo, and E. P. Mazzola, "Antileukemic Compounds Derived from the Chemical Modification of Macrocyclic Trichothecenes 2, Derivatives of Roridins A and H and Verrucarins A and J," *J. Med. Chem.*, **27**, 239-244 (1984).
61. B. B. Jarvis, C. S. Yatawara, S. L. Greene, and V. W. Vrudhula, "Production of Verrucarol," *Appl. Environ. Microbiol.*, **48**, 673-674 (1984).
62. G. A. Bean, T. Fernando, B. B. Jarvis, and B. Bruton, "The Isolation and Identification of Trichothecene Metabolites from Plant Pathogenic Strains of *Myrothecium roridum*," *J. Nat. Prod.*, **47**, 727-729 (1984).
63. B. B. Jarvis, V. M. Vrudhula, D. W. Dishong, and G. W. Gokel, "Synthesis and Binding Studies of Crown Ethers Bearing Pharmacophoric Groups: Epoxy-Lariat Crown Ethers," *J. Org. Chem.*, **49**, 2423-2427 (1984).
64. B. B. Jarvis, "Trichothecene Mycotoxins from the Higher Plant *Baccharis megapotamica*," in Toxigenic Fungi - Their Toxins and Health Hazard, H. Kurata and Y. Ueno (Eds.), Elsevier, New York, 1984, pp. 312-321.
65. B. B. Jarvis, N. B. Pena, M. M. Rao, N. S. Cömezoglu, and T. F. Cömezoglu, "Allelopathic Agents from *Parthenium hysterophorus* and *Baccharis megapotamica*," in The Chemistry of Allelopathy, A. C. Thompson (Ed.), ACS Symposium Series, Washington, D.C., No. 268, 1984, pp. 149-159.

66. F. S. Chu, G. S. Zhang, M. D. Williams, and B. B. Jarvis, "Production and Characterization of Antibody Against Deoxyverrucarol," *Appl. Environ. Microbiol.*, **48**, 781-784 (1984).
67. H. C. Cutler and B. B. Jarvis, "Preliminary Observations on the Effects of Macrocylic Trichothecenes on Plant Growth," *Exper. Environ. Bot.*, **25**, 115-128 (1985).
68. B. B. Jarvis, G. Pavanadasivam, and G. A. Bean, "Mycotoxin Production from *Myrothecium* Species," in *Trichothecenes and Other Mycotoxins*, J. Lacey (Ed.), John Wiley and Sons. Ltd., London, 1985, pp. 221-229.
69. B. B. Jarvis, Y.-W. Lee, C. S. Yatawara, D. B. Mazzocchi, J. L. Flippen-Anderson, R. Gilardi, and C. George, " 7α -Hydroxytrichodermol, A New Trichothecene from *Myrothecium roridum*," *Appl. Environ. Microbiol.*, **50**, 1225-1228 (1985).
70. B. B. Jarvis, Y.-W. Lee, F. T. Cömezoglu, S. N. Cömezoglu, and G. A. Bean, "Myrotoxins, A New Class of Macrocylic Trichothecenes," *Tetrahedron Lett.*, **26**, 4859-4862 (1985).
71. B. B. Jarvis, N. B. Pena, N. S. Cömezoglu, and M. M. Rao, "Nontrichothecenes from *Baccharis megapotamica*," *Phytochemistry*, **25**, 533-535 (1986).
72. T. Fernando, B. B. Jarvis, and G. Bean, "Effect of Microelements on the Production of Roridin E by *Myrothecium roridum*, a Strain Pathogenic to Muskmelon," *Trans. Br. Mycol. Soc.*, **86**, 273-277 (1986).
73. W. A. Croft, B. B. Jarvis, and C. S. Yatawara, "Airborne Outbreak of Trichothecene Toxicosis," *Atmospheric Environ.*, **20**, 549-552 (1986).
74. B. B. Jarvis, Y.-W. Lee, S. N. Yatawara, and C. S. Cömezoglu, "Trichothecenes Produced by *Stachybotrys atra* from Eastern Europe," *Appl. Environ. Microbiol.*, **51**, 915-918 (1986).
75. B. B. Jarvis and C. S. Yatawara, "Roritoxins, New Macrocylic Trichothecenes from *Myrothecium roridum*," *J. Org. Chem.*, **51**, 2906-2910 (1986).
76. B. B. Jarvis, F. T. Cömezoglu, Y.-W. Lee, J. L. Flippen-Anderson, R. D. Gilardi, and C. F. George, "Novel Macrocylic Trichothecenes from *Myrothecium roridum*," *Bull. Soc. Chim. Belg.*, **95**, 681-697 (1986).
77. B. B. Jarvis, "Trichothecene Mycotoxins and Their Interactions with Plants," in *Mycotoxins and Phycotoxins*, P. S. Steyn (Ed.), Elsevier, Amsterdam, 1986, pp. 153-160.
78. N. M. Ammar and B. B. Jarvis, "Major Flavonoids of *Tephrosia nubica*," *J. Nat. Prod.*, **49**, 719-720 (1986).

79. T. Fernando, B. B. Jarvis, and G. A. Bean, "A Comparison of the Effect of Three Fungicides on Growth and Roridin E Production by *Myrothecium roridum*," *Mycopathologia*, **95**, 155-158 (1986).
80. B. B. Jarvis, S. N. Cömezoglu, M. M. Rao, N. B. Pena, F. E. Boettner, Tara, M. Williams, G. Forsyth, and B. Epling, "Isolation of Macrocyclic Trichothecenes from a Large Scale Extract of *Baccharis megapotamica*," *J. Org. Chem.*, **52**, 45-56 (1987).
81. T. Krishnamurthy, E. W. Sarver, S. L. Greene, and B. B. Jarvis, "Mass Spectral Investigations on Trichothecene Mycotoxins II, Detection and Quantitative of Macrocyclic Trichothecenes by Gas Chromatography/Negative Ion Chemical Ionization Mass Spectroscopy," *J. Assoc. Off. Anal. Chemists*, **70**, 132-140 (1987).
82. T. Kommedahl, H. K. Abbus, C. J. Mirocha, G. A. Bean, B. B. Jarvis, and M.-D Guo, "Toxigenic *Fusarium* Species Found in Roots and Rhizospheres of *Baccharis* Species from Brazil," *Phytopathology*, **77**, 584-588 (1987).
83. B. B. Jarvis, K. M. Wells, Y.-W. Lee, G. A. Bean, T. Kommedahl, S. S. Barros, and C. S. L. Barros, "Macrocyclic Trichothecene Mycotoxins from Species of Brazilian *Baccharis*," *Phytopathology*, **77**, 980-984 (1987).
84. B. B. Jarvis, S. N. Cömezoglu, H. L. Ammon, C. K. Breedlove, R. F. Bryan, R. W. Miller, M. K. Woode, D. R. Streelman, A. T. Sneden, R. G. Dailey, Jr., and S. M. Kupchan, "New Macrocyclic Trichothecenes from *Baccharis megapotamica*," *J. Nat. Prod.*, **50**, 815-828 (1987).
85. W. G. Sorenson, D. G. Frazer, B. B. Jarvis, J. Simpson, and V. A. Robinson "Trichothecene Mycotoxins in Airborne Conidia of *Stachybotrys atra*," *Appl. Environ. Microbiol.*, **53**, 1370-1375 (1987).
86. G. A. Bean and B. B. Jarvis, "Mycotoxins Produced by *Myrothecium roridum*, a Fungus Pathogenic to Tomatoes," *Biodeterior. Res.*, **1**, 127-134 (1987).
87. E. P. Mazzola and B. B. Jarvis, "Structure and Characterization of Trichothecenes," in Recent Advances in Organic NMR Spectroscopy, J. B. Lambert and R. Rittner (Eds.), Norell Press, Landisville, NJ, 1987, pp. 153-162.
88. B. B. Jarvis, S. N. Cömozoglu, and M. E. Alvarez, "Oxidation Reactions of Baccharinoid B5," *J. Org. Chem.*, **53**, 1918-1922 (1988).
89. B. B. Jarvis, J. O. Midiwo, G. A. Bean, M. B. Aboul-Nasr, and C. S. Barros, "The Mystery of Trichothecene Antibiotics in *Baccharis* Species," *J. Nat. Prod.*, **51**, 736-744 (1988).
90. B. B. Jarvis, J. Kuti, and G. A. Bean, "Phytotoxicity of Macrocyclic Trichothecenes Toward *Baccharis* Cell Lines," *Proc. Jap. Assoc. Mycotox.*, Special Issue No. 1, 1988, K. Aibara, S. Kumagai, K. Ohtsubo, and T. Yoshizawa (eds.), pp. 199-203.

91. C. J. Mirocha, H. K. Abbas, T. Kommedahl, and B. B. Jarvis, "Mycotoxins Produced by *Fusarium oxysporum* and *F. sporotrichioides* Isolated from *Baccharis* Species from Brazil," *Appl. Environ. Microbiol.*, **55**, 254-255 (1989).
92. B. J. Hughes, G. C. Hsieh, B. B. Jarvis, and R. P. Sharma, "Effects of Macrocylic Trichothecene Mycotoxins on the Murine Immune System," *Arch. Environ. Contam. Toxicol.*, **18**, 388-395 (1989).
93. B. Yagen and B. B. Jarvis, "Synthesis of Tritium Labelled Verrucarol and Verrucaric Acid," *J. Label. Comp. Radiopharm.*, **27**, 675-681 (1989).
94. B. B. Jarvis, J. O. Midiwo, and M.-D. Guo, "12,13-Deoxytrichoverrins from *Myrothecium verrucaria*," *J. Nat. Prod.*, **52**, 663-665 (1989).
95. M. B. Aboul-Nasr, G. A. Bean, and B. B. Jarvis, "*Chlorella*, *Ustilago*, and *Trichoderma* Biological Assay for Detection of Macrocylic Trichothecenes," *Biodeterior. Res.*, **2**, 363-369 (1989).
96. J. O. Kuti, N. Mokhtari, B. B. Jarvis, and G. A. Bean, "Allelopathic Potential of Plant-Derived Macrocylic Trichothecenes on Selected Crop Species," *Biodeterior. Res.*, **2**, 383-392 (1989).
97. H. B. Schiefer, D. S. Hancock, and B. B. Jarvis, "Toxicology of Novel Macrocylic Trichothecenes Baccharinoid B4, Myrotoxin B and Roritoxin B," *J. Vet. Med.*, **A36**, 152-160 (1989).
98. B. B. Jarvis, M. E. Alvarez, G. Wang, and H. L. Ammon, "Solvolytic Cyclization of 4,15-Anhydroverrucarol. A Facile Trichothecene-10,13-Cyclotrithothecene Rearrangement," *J. Org. Chem.*, **54**, 4493-4494 (1989).
99. T. Krishnamurthy, D. J. Beck, R. K. Isensee, and B. B. Jarvis, "Mass Spectral Investigations on Trichothecene Mycotoxins. VII. LC-Thermospray Mass Spectrometric Analysis of Macrocylic Trichothecenes," *J. Chromat.*, **469**, 209-222 (1989).
100. B. B. Jarvis, "Mycotoxins - An Overview," in *Natural Toxins*, C. L. Ownby and G. V. Odell (eds.), Pergamon Press, New York, 1989, pp. 17-29.
101. B. B. Jarvis, G. A. Bean, J. O. Midiwo, M. B. Aboul-Nasr, J. Kuti, and N. Mokhtari, "The Mystery of Trichothecenes in Higher Plants," *Mycotoxins and Phycotoxins '88*, S. Natorio, K. Hashimoto, and Y. Ueno (eds.), Elsevier, Amsterdam, 1989, pp.197-204.
102. B. B. Jarvis and A. M. Acierto, "Anticancer Properties of Trichothecenes," in *Trichothecene Mycotoxicosis: Pathophysiologic Effects*. Vol. I, V. R. Beasley (ed.), CRC Press, Boca Raton, FL, 1989, pp.73-105.

103. B. B. Jarvis, "Mycotoxins and Indoor Air Quality," in **Biological Contaminants in Indoor Environments**, AST STP 1071, P. R. Morey, J. C. Feely, Sr., and J. A. Otten (eds.), American Society for Testing and Materials, Philadelphia, 1990, pp. 201-214.
104. B. B. Jarvis, D. B. Mazzocchi, H. L. Ammon, E. P. Mazzola, J. L. Flippen-Anderson, R. D. Gilardi, and C. F. George, "Conformational Effects in Trichothecenes: Structures of 15-Hydroxy C4 and C15 Ketones," *J. Org. Chem.*, **55**, 360-362 (1990).
105. B. B. Jarvis, C. A. Armstrong, and M. Zeng, "Use of Resins for Trichothecene Production in Liquid Cultures," *J. Antibiotics*, **43**, 1502-1504 (1990).
106. J. O. Kuti, B. B. Jarvis, N. Mokhtari-Rejali, and G. A. Bean, "Allelochemical Regulation of Reproduction and Seed Germination of Two Brazilian *Baccharis* Species by Phytotoxic Trichothecenes," *J. Chem. Ecol.* **16**, 3441-3453 (1990).
107. B. B. Jarvis, K. M. Wells, and T. Kaufmann, "A New Synthetic Route to α -Hydroxybutyrolactones," *Synthesis*, 1079-1082 (1990).
108. B. B. Jarvis, M. Zeng, and E. P. Mazzola, "Novel Rearrangements of Macrocyclic Trichothecenes," *Tetrahedron Lett.*, **31**, 4401-4404 (1990).
109. B. J. Hughes, B. B. Jarvis, and R. P. Sharma, "Effects of Macrocyclic Trichothecene Congeners on the viability and Mitogenesis of Murine Splenic Lymphocytes," *Toxicol. Lett.*, **50**, 57-67 (1990).
110. B. B. Jarvis, R. O. Kollah, and M. Zeng, "Chemical Modifications of the Macrocyclic Trichothecenes Baccharinoid B4 and Myrotoxin B," *Quimica Nova*, **13**, 315-326 (1990).
111. A. C. Morgan, Jr., F. T. Comezoglu, R. Manger, B. B. Jarvis, P. G. Abrams, and G. Sivam, "Immunoconjugates of a Protein Synthesis-inhibiting Drug," in **Therapeutic Monoclonal Antibodies**, C. A. K. Borrebaeck and J. W. Larrick (Eds.), Stockton Press, New York, 1990, pp. 143-158.
112. B. B. Jarvis, N. Mokhtari-Rejali, E. P. Schenkel, N. I. Matzenbacher, and C. S. Barros, "Trichothecene Mycotoxins from Brazilian *Baccharis* Species," *Phytochemistry*, **30**, 789-797 (1991).
113. O. M. O. El Maghraby, G. A. Bean, B. B. Jarvis, and M. B. Aboul-Nasr, "Macrocyclic Trichothecenes Produced by *Stachybotrys* Isolates from Egypt and Eastern Europe," *Mycopathologia*, **113**, 109-115 (1991).
114. B. B. Jarvis, F. T. Comezoglu, S. Wang, and H. L. Ammon, "Myrotoxins from a Plant Pathogenic Isolate of *Myrothecium roridum*," *Mycotoxin Res.*, **7**, 73-78 (1991).
115. B. B. Jarvis, "Macrocyclic Trichothecenes," in **Mycotoxins and Phytoalexins in Human and Animal Health**, R. P. Sharma and D. K. Salunkhe (Eds.), CRC Press, 1991, pp. 361-421.

116. G. A. Bean, B. B. Jarvis, and M. B. Aboul-Nasr, "A Biological Assay for the Detection of *Myrothecium* spp.-produced Macrocyclic Trichothecenes," *Mycopathology*, **119**, 175-180 (1992).
117. F. Bergmann, B. Yagen, and B. B. Jarvis, "The Toxicity of Macrocyclic Trichothecenes Administered Directly into the Rat Brain," *Toxicol.*, **30**, 1291-1294 (1992).
118. B. B. Jarvis, T. DeSilva, J. B. McAlpine, S. J. Swanson, and D. N. Whitten, "New Trichoverroids from *Myrothecium verrucaria* Isolated by High Speed Countercurrent Chromatography," *J. Nat. Prod.* **55**, 1441-1446 (1992).
119. J. O. Kuti and B. B. Jarvis, "Growth Hormone-like Activities in *In Vitro* Callus Induction and Growth of Four *Baccharis* species," *J. Plant Growth Reg.* **11**, 149-154 (1992).
120. Y.-N. Lin, B. B. Jarvis, and W. J. Bailey, "Synthesis and Polymerization of Bis Spiro Orthocarbonate Monomers," *Polymer Preprints*, **33**, 1111-1112 (1992).
121. Y.-N. Lin and B. B. Jarvis, "Synthesis and Polymerization of 12,13-Benzo-2,9-dimethylene-6-methyl-1,4,8,11-tetraoxadispiro[4.1.4.2]tridecane," *Polymer Preprints*, **33**, 1113-1114 (1992).
122. B. B. Jarvis, "Macrocyclic Trichothecenes from Brazilian *Baccharis* Species: from Microanalysis to Large Scale Isolation," *Phytochem. Anal.*, **3**, 241-249 (1992).
123. E. Johannig, P. R. Morey, and B. B. Jarvis, "Clinical-Epidemiological Investigation of Health Effects Caused by *Stachybotrys atra* Building Contamination," *Indoor Air '93*, **1**, 225-230 (1993).
124. S. L. Coon, S. Kotob, B. B. Jarvis, W. C. Fuqua, and R. M. Weiner, "Homogentisic Acid is the Product of the melA Gene Product Mediating Melanogenesis in the Marine Bacterium, Shewanella colwelliana D," *Appl. Environ. Microbiol.*, **60**, 3006-3010 (1994).
125. B. B. Jarvis, J. Salemme, and A. Morais, "*Stachybotrys* Toxins. 1," *Natural Toxins*, **3**, 10-16 (1995).
126. B. B. Jarvis, "Secondary Metabolites and their Role in Evolution," *An. Acad. bras. Ci.*, **67** (Supl. 3), 329-345 (1995).
127. B. B. Jarvis, S. Wang, C. Cox, M. M. Rao, V. Philip, M. S. Varaschin, and C. S. Barros, "Brazilian *Baccharis* Toxins: Livestock Poisoning and the Isolation of Macrocyclic Trichothecene Glucosides," *Natural Toxins*, **4**, 58-71 (1996).
128. B. B. Jarvis, S. Wang, and H. L. Ammon, "Trichoverroid Isomers," *J. Nat. Prod.*, **59**, 254-261 (1996).

129. E. Johanning, R. Biagini, D. Hull, P. Morey, B. Jarvis, and P. Landsbergis, "Health and Immunology Study Following Exposure to Toxigenic Fungi (*Stachybotrys chartarum*) in a Water-damaged Office Environment," *Int. Arch. Occup. Environ. Health*, **68**, 207-218 (1996).
130. S. Hashmi, L. R. Krusberg, and B. B. Jarvis, "Hatching Factor(s) in Corn Seedling Leachates for the Eggs of *Heterodera zea*," *Can. J. Zool.*, **74**, 1542-1546 (1996).
131. B. B. Jarvis, Y. Zhou, J. Jiang, S. Wang, W. G. Sorenson, E.-L. Hintikka, M. Nikulin, P. Parikka, R. A. Etzel, and D. G. Dearborn, "Toxigenic Molds in Water-Damaged Buildings: Dechlorogriseofulvins from *Memnoniella echinata*," *J. Nat. Prod.*, **59**, 553-554 (1996).
132. K. Reijula, M. Nikulin, B. B. Jarvis, and E.-L. Hintikka. "Stachybotrys atra-Induced Lung Injury." *Proc. 7th Internat. Conf. Indoor Air Quality and Climate - Indoor Air '96*, **3**: 639-643 (1996).
133. M. Nikulin, K. E. Reijula, B. B. Jarvis, and E.-L. Hintikka, "Experimental Lung Mycotoxicosis in Mice Induced by *Stachybotrys atra*," *Internat. J. Exp. Pathol.*, **77**, 213-218 (1996).
134. M. Nikulin, K. E. Reijula, B. B. Jarvis, P. Veijalainen, and E.-L. Hintikka, "Effects of Internasal Exposure to Spores of *Stachybotrys atra* in Mice," *Fundam. Appl. Toxicol.*, **35**, 182-188 (1997).
135. S. C. Trapp, B. B. Jarvis, and T. M. Hohn, Characterization of the Macrocyclic Trichothecene Pathway Gene Cluster in *Myrothecium roridum*. *Mol. Gen. Genetics*, *Mol. Gen. Genet.* **257**, 421-432 (1998).
136. M. J. Hodgson, P. Morey, W.-Y. Leung, L. Morrow, J. D. Miller, B. B. Jarvis, H. Robbins, J. F. Halsey, and E. Storey. Building-associated pulmonary disease from exposure to *Stachybotrys chartarum* and *Aspergillus versicolor*. *J. Occup. Environ. Med.*, **40**, 241-249 (1998).
137. R. A. Etzel, E. Montana, W. G. Sorenson, G. J. Kullman, T. M. Allen, D. R. Olson, B. B. Jarvis, J. D. Miller, and D. G. Dearborn. Acute Pulmonary Hemorrhage in Infants Associated with Exposure to *Stachybotrys atra* and Other Fungi. *Arch. Pediatr. Adolesc. Med.*, **152**, 757-762 (1998).
138. B. B. Jarvis, W. G. Sorenson, E.-L. Hintikka, M. Nikulin, Y. Zhou, J. Jiang, S. Wang, S. Hinkley, R. A. Etzel, and D. Dearborn. "Study of Toxin Production by Isolates of *Stachybotrys chartarum* and *Memnoniella echinata* isolated During a Study of Pulmonary Hemosiderosis in Infants," *Appl. Environ. Microbiol.*, **64**, 3620-3625 (1998).
139. M. S. Varaschin, C. S. L. Barros, B. B. Jarvis. Intoxicação experimental por *Baccharis coridifolia* (Compositae) em bovinos. *Pesq. Vet. Bras.*, **18**, 69-75 (1998).
140. E. Johanning, M. Gareis, E.L. Hintikka, C. S. Yang, M. Nikulin, B. B. Jarvis, and R.

Dietrich. "Toxicity Screening of Fungal Samples: Results of Sentinel Health Investigations Related to Indoor *Stachybotrys atra* (*chartarum*) Exposure." *Mycotoxin Research*, **14**, 60-73 (1998).

141. S. F. Hinkley, J. Jiang, E. P. Mazzola, and B. B. Jarvis, "Atranones: Novel Diterpenoids from the Toxicogenic Mold *Stachybotrys atra*," *Tetrahedron Lett.*, **40**, 2725-2728 (1999).
142. M.-Y. Lee, S. Li, B. B. Jarvis, and J. J. Pestka, "Effects of Satratoxins and other Macrocyclic Trichothecenes on IL-2 Production and Viability of EL-4 Thymoma Cells." *J. Toxicol. Environ. Health, Part A*, **57**, 459-474 (1999).
143. S. F. Hinkley, J. C. Fettinger, K. Dudley, and B. B. Jarvis, "Memnobotrins and Memnoconols: Novel Metabolites from *Memnoniella echinata*," *J. Antibiotics*, **52**, 988-997.
144. S. Vesper, D. G. Dearborn, I. Yike, T. Allen, J. Sobolewski, S. F. Hinkley, B. B. Jarvis, and R. A. Haugland, "Evaluation of *Stachybotrys chartarum* in the House of an Infant with Pulmonary Hemorrhage: Quantitative Assessment before, during, and after Remediation," *J. Urban Health*, in press.
145. G.-Y. Yang, B. B. Jarvis, and J. J. Pestka, "Apoptosis Induction by the Satratoxins and Other Trichothecene Mycotoxins: Relationship to ERK, p38 MARK and SAPK/JNK Activation." *Tox. Appl. Pharm.*, in press.
146. S. F. Hinkley, E. P. Mazzola, J. C. Fettinger, Y.-F. Lam, and B. B. Jarvis, "Atranones A-G: A unique series of metabolites from the toxicogenic mold *Stachybotrys atra*." *Phytochemistry*, in press.
147. S. F. Hinkley, J. Moore, J. Squillari, K. Dudley and B. B. Jarvis, "New Compounds and Isolation Artifacts from the Fungus *Stachybotrys atra*." *J. Nat. Prod.*, submitted.

1. Books

1. B. B. Jarvis and P. H. Mazzocchi, "Form and Function, An Organic Chemistry Module," Harper and Row Publishers, Evanston, Illinois(1973), 2nd Edition, 1978
2. B. B. Jarvis (Unit Director for), "Chemcom: Chemistry and Health," American Chemical Society, Washington, D.C. 1986.

2. Contracts and Grants (1983-present)

1. NIH-NCI-CA-25967, "New and Potent Anticancer Compounds," December 1, 1983-November 30, 1986. \$254,290 (Direct costs only).
2. U.S. Army Contract DAMD17-82-C-2240, "Trichothecene Mycotoxins: Preparation, Analysis, and Chemical Reactivity," September 1 1982-August 31, 1985. \$336,782.

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3. U.S. Army Contract DAMD17-85-C-5129, "Preparation of Radiolabeled Macrocyclic Trichothecenes, Simple Trichothecenes for Generation of Generic Antibodies, and a Study of the Fate of Trichothecenes in Soil," Sept. 1, 1985-Nov. 14, 1986. \$132,844.
4. NIH-NCI-CA-25967, "Chemistry of the Macrocyclic Trichothecene Antibiotics," December 1, 1986-November 30, 1989. \$207,610 (Direct costs only).
5. NSF INT-86-10994, "Interaction of Trichothecene - Producing Fungi with Higher Plants," July 15, 1986-June 30, 1989. \$9,406.
6. NeoRx Corporation Contract, "Preparation of Trichothecene Derivatives for Conjugation to Antibodies," August 1, 1986-April 30, 1989. [REDACTED]
7. Agricultural Biotechnology Center Small Grant Award, "Plant Produced Fungal Toxins," October 1, 1987-June 30, 1988. [REDACTED] Co-PI with G. A. Bean).
8. NeoRx Corporation Contract, "Preparation of Trichothecene Derivatives for Conjugation to Antibodies," May 1, 1989-April 30, 1990; [REDACTED] 3.
9. NIH-GM-43724, "Chemistry and Biology of the Macrocyclic Trichothecenes," January 1, 1990-December 31, 1995. \$411,423 (Direct costs only).
10. NSF-INT-90-09433, "U.S. - Brazil (STI) Workshop on Natural Products Chemistry," April 1, 1990 - March 31, 1991, [REDACTED] (Total Costs).
11. Maryland Industrial Partnership Contract, "Monomers That Expand Upon Curing for Use in the Electric Industry," June 1, 1990 - May 31, 1991, [REDACTED] (Total Costs).
12. UMCP Biomedical Research Support Award, "Countercurrent Chromatography," June 1, 1990 - March 31, 1991, [REDACTED] (Co-PI with R. Armstrong, J. Gerlt, and J. Kozarich).
13. NIH-R25-GM-50069, "Biomedical Minority Access Program," Oct. 1, 1993-Sept. 30, 1995, \$318,750 (Total Costs; Co-PI with W. Higgins).
14. UMCP General Research Board Research Equipment Award, "Upgrade of Mass Spectrometry Data Handling System," July 1, 1994-June 30, 1995.
15. NIH-NCI Contract: "Preparation of 8 β -Hydroxy-9 β ,10 β -epoxyroridin A," August 1, 1995-July 31, 1996, \$5200.
16. NSF "Major Equipment Grant for Purchase of 400 MHz and Upgrade of 500 MHz NMR Instruments," January 1, 1996-December 31, 1997, \$380,000.
17. NIH-NCI Contract: "Preparation of 8 β -Hydroxy-9 β ,10 β -epoxyroridin A and 8 β -Hydroxy-9 β ,10 β -epoxyverrucarin A " March 15, 1996-March 14, 1997, \$7000.

18. EPA Contract, "Interstitial Lung Disease: Moisture, Molds, and Buildings," \$50,000 subcontract, total contract: \$572,710, December 2, 1996 - December 1, 1999.
19. Center for Indoor Air Research Grant, "Development of Analytical Methods to Measure Levels of Fungal Toxins in Indoor Air," 01/01/98 - 12/31/00, [REDACTED]
20. U.S. Army Contract DI-MGMT-80227, "FT-NIR Rapid Determination of Food Integrity," 06/01/98 - 05/31/00, \$236,668 (co-PI E. Calvey).
21. NSF "Major Equipment Grant for Purchase of CCD and Powder Diffraction X-Ray Spectrometers and Upgrade of Rigaku D-MAX B Theta-theta Powder Diffractometer," September 1, 1998-August 31, 2000, \$265,330.

3. Special Achievements

- a) Invited as a Speaker for American Chemical Society Speaking Tour (1982-84).
 - b) ACS Short Course on "Medium Effects, Crown Ethers, and Phase Transfer Catalysis in Organic Synthesis" This course is now offered as an ACS Audio Course.
 - c) Vice-chairman, Gordon Research Conference on Mycotoxins, June 1985.
 - d) Chairman, GRC on Mycotoxins and Phycotoxins, June 1987.
 - e) GRB Semester Research Award (Fall 1985).
 - f) Maryland Sigma Xi Award for Outstanding Research, 1987.
 - g) Elected AAAS Fellow, January 1989.
 - h) University Teacher-Scholar Awardee, 1991-92.
 - i) Gulf Coast Lecture Tour Speaker for Am. Chem. Society (Oct. 26-30, 1992).
 - j) Indiana Lecture Tour Speaker for American Chemical Society (April 12-16, 1993)
 - k) Northeast Lecture Tour Speaker for Am. Chem. Society (November 15-19, 1993).
 - l) Member of *ChemTracks* Editorial Board
 - m) Member of the Editorial Board of *Natural Toxins* (1998-Present)
- 4) Invited Seminars: (1983-present)
1. Shanghai Institute of Organic Chemistry, China, August 23, 1983.
 2. Medical Institute of the Chinese Academy of Medicine, Beijing, China, August 25, 1983.

000364

3. Institute of Microbioal Chemistry, Tokyo, Japan, August 31, 1983.
4. Stevens Institute of Technology, March 2, 1984.
5. Polish Academy of Sciences, Posnan, Poland, August 11, 1984.
6. Hungarian Academy of Sciences, Budapest, Hungary, August 16, 1984.
7. University of Virginia, April 3, 1985.
8. American Cyanamide, Princeton, NJ, November 6, 1985.
9. University of Virginia, Charlottesville, VA, March 2, 1986.
10. Ecole Polytechnique, Paris, France, August 12, 1986.
11. LaSalle University, Philadelphia, PA, November 11, 1986.
12. Manhattan College, Riverdale, NY, November 12, 1986.
13. Frostburg State College/Western Maryland Local ACS, Nov. 16, 1987.
14. Morgan State College, Baltimore, MD, February 5, 1988.
15. Department of Horticulture, UMCP, February 22, 1988.
16. NeoRx Corporation, Seattle, WA, February 26, 1988.
17. University of California-Santa Barbara, March 2, 1988.
18. University of California-Irvine, March 4, 1988.
19. University of Delaware, March 16, 1988.
20. Annual Sigma Xi Meeting, UMCP, March 25, 1988.
21. University of Iowa, April 4, 1988.
22. University of Sao Paulo, May 9, 1988.
23. Iowa State University, August 4, 1988.
24. Shionogi Pharmaceutical Company, August 23, 1988.
25. University of Mississippi, November 17, 1988.
26. University of Puerto Rico, March 27, 1989.
27. Federal University Rio du Sul, Porto Alegre, Brazil, May 5, 1989.
28. Chemical Society of Washington, November 9, 1989.
29. UMBC, March 27, 1990.
30. Abbott Laboratories, April 3, 1990.
31. University of Sao Paulo, June 1, 1990.
32. Walter Reed Medical Center, March 15, 1991.
33. U.S. State Department, April 17, 1991.
34. University of California at Irvine, April 25 and 26, 1991.
35. Organon, Inc., Rockville, MD, September, 10, 1991.
36. University of Westchester, PA, September 11, 1991.
37. Shippensburg University, PA, November 20, 1992.
38. University of Delaware, March 3, 1993.
39. Frederick Cancer Research Center, Frederick, MD, March 19, 1993.
40. University of Kenyatta, Nairobi, Kenya, June 15, 1993.
41. National Institutes of Health, Bethesda, MD, June 25, 1993.
42. Washington College, Chestertown, MD, November 10, 1993
43. Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT, November 18, 1993
44. Ohio Wesleyan University, Delaware, Ohio, March 3, 1994
45. Denison University, Granville, Ohio, March 4, 1994
46. Gettysburg College, Gettysburg, PA, November 1994
47. Junior Sciene & Humanities Symposium, Greenbelt, MD, March 16, 1995
48. University of Sao Paulo, Sao Carlos, Brazil, April 24, 1995

49. University of Sao Paulo, Campinas, Brazil, April 25, 1995
50. Vanderbilt University, September 22, 1995
51. Washington DC section of the American Chemical Society, May 9, 1996
52. Western Maryland College, Oct. 9, 1998.
53. Danish Technical University, Sept. 14, 1999.
54. University of Copenhagen, Oct. 28, 1999.
55. University of Munich, Nov. 18, 1999

Book Chapters

1. B. B. Jarvis, R. M. Eppley and E. P. Mazzola, "Chemistry and Bioproduction of Macrocyclic Trichothecenes," in Trichothecenes - Chemical Biological and Toxicological Aspects, Y. Ueno (Ed.), Kodansha Scientific, Tokyo, 1983.
2. B. B. Jarvis, "Trichothecene Mycotoxins from the Higher Plant *Baccharis megapotamica*," in Toxogenic Fungi - Their Toxins and Health Hazard, H. Kurata and Y. Ueno (Eds.), Elsevier, New York, 1984, pp. 312-321.
3. B. B. Jarvis, N. B. Pena, M. M. Rao, N. S. Cömezoglu, and T. F. Cömezoglu, "Allelopathic Agents from *Parthenium hysterophorus* and *Baccharis megapotamica*," in The Chemistry of Allelopathy, A. C. Thompson (Ed.), ACS Symposium Series, Washington, D.C., No. 268, 1984, pp. 149-159.
4. B. B. Jarvis, G. Pavanadasivam, and G. A. Bean, "Mycotoxins Production from *Myrothecium* Species," in Trichothecenes and Other Mycotoxins, J. Lacey (Ed.), John Wiley and Sons, Ltd., London, 1985, pp. 221-229.
5. B. B. Jarvis, "Trichothecene Mycotoxins and their Interactions with Plants," in Mycotoxins and Phycotoxins, P. S. Steyn (Ed.), Elsevier, Amsterdam, 1986, pp. 153-160.
6. E. P. Mazzola and B. B. Jarvis, "Structure and Characterization of Trichothecenes," in Recent Advances in Organic NMR Spectroscopy, J. B. Lambert and R. Rittner (Eds.), Norell Press, Landisville, NJ, 1987, pp. 153-162.
7. B. B. Jarvis and A. M. Acierto, "Anticancer Properties of Trichothecenes," in Pathophysiology of Trichothecenes, V. Beasley (Ed.), CRC Press, Boca Raton, FL, 1989, pp. 73-105.
8. B. B. Jarvis, "Mycotoxins - An Overview," in Animal, Plants, and Microbial Toxins, C. L. Ownby and G. V. Odell (Eds.), Pergamon Press, Oxford, 1989, pp. 17-29.
9. B. B. Jarvis, G. A. Bean, J. O. Midiwo, M. B. Aboul-Nasr, J. Kuti, and N. Mokhtari, "The Mystery of Trichothecenes in Higher Plants, in Proceedings of the 7th International Symposium on Mycotoxins and Phycotoxins, Y. Ueno (Ed.), Elsevier, Amsterdam, 1989, pp. 197-204.

000366

10. B. B. Jarvis, J. O. Kuti, and G. A. Bean, "Phytotoxicity of Macrocyclic Trichothecenes toward *Baccharis* Cell Lines, in Proceeding of the 5th International Plant Pathology Congress, T. Yoshizawa (Ed.), Elsevier, Amsterdam, 1989, pp. 199-203.
 11. B. B. Jarvis, "Mycotoxins and Indoor Air Quality," in Biological Contaminants in Indoor Environments, AST STP 1071, P. R. Morey and J. C. Feely, Sr. (Eds.), American Society for Testing and Materials, Philadelphia, 1990, pp. 201-214.
 12. A. C. Morgan, Jr., F. T. Comezoglu, R. Manger, B. B. Jarvis, P. G. Abrams, and G. Sivam, "Immunoconjugates of a Protein Synthesis-inhibiting Drug," in Therapeutic Monoclonal Antibodies, C. A. K. Borrebaeck and J. W. Larrick (Eds.), Stockton Press, New York, 1990, pp. 143-158.
 13. B. B. Jarvis, "Macrocyclic Trichothecenes," in Mycotoxins and Phytoalexins, R. P. Sharma and D. K. Salunkhe (Eds.), CRC Press, 1991, pp. 361-421.
 14. B. B. Jarvis, "Mycotoxins in the Air: Keep Your Buildings Dry or the Bogeyman Will Get You," in Fungi and Bacteria in Indoor Air Environments, E. Johanning and C. S. Yang (Eds.), Eastern New York Occupational Health Program, Latham, NY, 1995, pp. 35-44.
 15. B. B. Jarvis, S. C. Trapp, and T M. Hohn, "Fungal Toxins Produced by Brazilian *Baccharis* Species: a Case for Horizontal Gene Transfer?" in Proceedings of the International Compositae Conference, KEW 1994, Vol. 2, Biology & Utilization, P. D. S. Caligari and D. J. N. Hind (Eds.), Royal Botanic Gardens, Kew, 1996, pp. 261-267.
 16. B. B. Jarvis and J. D. Miller, "Natural Products, Complexity, and Evolution," in Recent Advances in Phytochemistry: Phytochemical Diversity and Redundancy in Ecological Systems, Vol. 30, J. T. Romeo, J. A. Saunders, and P. Barbosa (eds.), Plenum, New York, NY, pp. 265-293 (1996).
 17. B. B. Jarvis and S. Hinkley, "Analysis for *Stachybotrys* Toxins," in Bioaerosols, Fungi, and Mycotoxins, E. Johanning and C. S. Yang (eds.), Boyd Printing, Albany, NY, pp. 161-177 (2000).
 18. S. Hinkley and B. B. Jarvis, "Method for *Stachybotrys* Toxins," in Methods in Molecular Biology: The Mycotoxin Protocols, Humana Press, in press.
 19. B. B. Jarvis, "Role of Natural Products in Evolution, in Advances in Phytochemistry: The Evolution of Metabolic Pathways, Vol. 34, in press.
2. A. Papers Presented at Professional Meetings (1983 - Present)
 1. F. E. Boettner, B. B. Jarvis, and N. B. Pena, "Macrocyclic Trichothecenes from a Large Scale Extract of *Baccharis megapotamica*," 186th National Meeting of the ACS, Washington, D.C., August 29, 1983.

2. B. B. Jarvis, J. O. Midiwo, N. B. Pena, N. S. Cömezoglu and F. T. Cömezoglu, "Antileukemic Macrocyclic Trichothecenes," 35th Southeast Regional Meeting of the ACS, Charlotte, NC, November 9, 1983.
3. P. J. Hannan, A. V. Stiffey, B. B. Jarvis, and S. L. Greene, "Reaction of Trichothecenes with Vegetation as a Factor in their Concentration in Field Samples," 188th National Meeting of the ACS, Philadelphia, PA, August 27, 1984.
4. B. B. Jarvis, T. F. Cömezoglu, and N. S. Cömezoglu, "Oxidation and Rearrangements of Macrocyclic Trichothecenes," 188th National Meeting of The ACS, Philadelphia, PA, August 27, 1984.
5. B. B. Jarvis, "Oxidative Rearrangements," International Symposium on Advances in Methods of Organic Synthesis, Jadwisin, Poland, July 5, 1984.
6. B. B. Jarvis and G. A. Bean, "Chemical Interactions Between Fungi and Higher Plants," 14th International IUPAC Symposium on the Chemistry of Natural Products, Posnan, Poland, July 12, 1984.
7. G. A. Bean and B. B. Jarvis, "Allelopathic Trichothecene Compounds Produced by Fungus *Myrothecium*," 190th National Meeting of the American Chemical Society, Chicago, IL, September 10, 1985.
8. B. B. Jarvis, T. F. Cömezoglu, M. E. Alvarez, and D. B. Mazzocchi, "Correlation of Chemical Reactivity with Biological Activity for the Trichothecenes," 19th Middle Atlantic ACS Meeting, Monmouth College, West Long Branch, NJ, May 22, 1985.
9. B. B. Jarvis and C. S. Yatawara, "Isolation of New Mycotoxins from *M. Roridum*," 191st National ACS Meeting, New York City, April 15, 1986.
10. B. B. Jarvis, J. S. Rinker, and M. L. Franklin, "Toxin Production from *S. atra*," 20th Middle Atlantic ACS Meeting, Baltimore, MD, September 3, 1986.
11. B. B. Jarvis, J. O. Midiwo, and R. N. Ominde, "Chemical Modifications of Macrocyclic Trichothecenes," 20th Middle Atlantic ACS Meeting, Baltimore, MD, September 3, 1986.
12. B. B. Jarvis and M. Zeng, "Chemical Modifications of Myrotoxins," 20th Middle Atlantic ACS Meeting, Baltimore, MD, September 3, 1986.
13. B. B. Jarvis, M. E. Alvarez, and D. B. Mazzocchi, "Correlation of Chemical and Biological Reactivities of the Trichothecenes," 20th Middle Atlantic ACS Meeting, Baltimore, MD, September 3, 1986.

000368

14. B. B. Jarvis, M. E. Alvarez, D. B. Mazzocchi, and F. T. Cömezoglu, "Mechanisms for the Rearrangements of Trichothecenes," 194th National ACS Meeting, New Orleans, September 3, 1987.
15. G. A. Bean and B. B. Jarvis, "Role of Trichothecenes in Plant Pathogenicity," 2nd Meeting of the Pan-American Biodeterioration Society, Washington, D.C., July 30, 1988.
16. G. Sivam, F. T. Comezoglu, R. Manager, M. A. Gray, B. B. Jarvis, and A. C. Morgan, "Immunoconjugates of Trichothecenes and Monoclonal Antibody," 4th International Conference on Monoclonal Antibody Immunoconjugates for Cancer, UCSD Cancer Center, San Diego, CA, March 31, 1989.
17. G. Sivam, F. T. Comezoglu, V. M. Vrudhula, K. Riker, M. A. Gray, A. Srinivasan, B. B. Jarvis, and A. C. Morgan, "Immunoconjugates of Small Molecule Protein Synthesis Inhibitor (Trichothecene) - an Update," International Symposium on Natural Toxins, Nanning, China, May 23, 1989.
18. G. Sivam, F. T. Comezoglu, V. M. Vrudhula, K. Riker, M. A. Gray, A. Srinivasan, B. B. Jarvis, and A. C. Morgan, "Immunoconjugates of Small Molecule Protein Synthesis Inhibitor (Trichothecene) - an Update," International Symposium on Natural Toxins, Nanjing, China, May 23, 1989.
19. B. B. Jarvis, N. Mokhtari-Regali, N. Aboul-Nasr, J. O. Kuti, and G. A. Bean, "Trichothecene Mycotoxins from Brazilian *Baccharis* Plants," 31st American Society of Pharmacognosy Annual Meeting, San Juan, Puerto Rico, August 1989.
20. Y.-N. Lin, B. B. Jarvis, and W. J. Bailey, "Synthesis and Polymerization of Bis Spiro Orthocarbonate Monomers," 203rd ACS National Meeting, San Fransico, CA, 4/92.
21. Y.-N. Lin and B. B. Jarvis, "Synthesis and Polymerization of 12,13-Benzo-2,9-dimethylene-6-methyl-1,4,8,11-tetraoxadispiro[4.1.4.2]tridecane," 203rd ACS National Meeting, San Fransico, CA, April 1992.
22. B. B. Jarvis, J. Salemme, A. Morais, B. Westfall, and F. Habteselasle, "Isolation and Characterization of *Stachybotrys atra* Toxins," 33rd American Society of Pharmacognosy Annual Meeting, Williamsburg, VA, July 1992.
23. B. B. Jarvis and S. Wang, "Isolation and Characterization of New Trichoveroids from a Complex Fermentation Mixture by Use of Countercurrent Chromatography," 204th ACS National Meeting, Washington, D. C., August 1992.
24. P. DeShong, B. B. Jarvis, and T. C. O'Haver, "Use of the CACHe System in Undergraduate Education. An Integrated, Multiyear Approach," 204th ACS National Meeting, Washington, D. C., August 1992.

25. B. B. Jarvis, S. Wang, H. L. Ammon, B. Westfall, and F. Habteselasle, "Biosynthetic Relationship between the Trichoveroids and the Four Diastereomeric Roridin E's," 44th Southeastern-26th Middle Atlantic Regional Meeting of the ACS, Crystal City, VA, December 7, 1992.
26. B. B. Jarvis, J. Salemme, A. Morais, B. Westfall, and F. Habteselasle, "Isolation and Characterization of *Stachybotrys atra* Toxins," 44th Southeastern-26th Middle Atlantic Regional Meeting of the ACS, Crystal City, VA, December 7, 1992.
27. E. Johanning, P. R. Morey, and B. B. Jarvis, "Clinical-epidemiological Investigation of Health Effects Caused by *Stachybotrys atra* Building Contamination.," Indoor Air '93, Helsinki, Finland, July 5, 1993.
28. S. C. Trapp, B. B. Jarvis, and T. M. Hohn, "Isolation and Nucleotide Sequence of the Trichodiene Synthase Gene from *Myrothecium roridum*," Host - Fungus Pathogenic Interactions, Keystone Symposium, Taos, New Mexico, February 27, 1995.
29. B. B. Jarvis, S. C. Trapp, and T. M. Hohn, "Relationship of Plant and Fungal Secondary Metabolite Genes," 18th Fungal Genetics Conference, Pacific Grove, CA, March 25, 1995.
30. W. G. Sorenson, B. B. Jarvis, J. Jiang, Y. Zhou, S. Wang, M. Nikulin, and E.-L. Hintikka. "Toxine im Zusammenhang mit *Stachybotrys* und *Memnoniella* in Haeusern mit Wasserschaden". 18th Mykotoxin Workshop. Kulmbach, Germany, June 11, 1996.

B. Invited Talks at Symposia (1983 - Present)

1. B. B. Jarvis, G. Pavanadasivam, and G. A. Bean, "Mycotoxin Production from *Myrothecium Species*," Mycotoxin Symposium in the 4th International Congress of Plant Pathology, University of Sidney, Sidney, Australia, August 12-15, 1983.
2. B. B. Jarvis, "New Trichothecenes from *Baccharis*, *Myrothecium* and *Stachybotrys Species*," Third International Mycological Congress, Tokyo, Japan, August 28-September 3, 1983.
3. B. B. Jarvis and G. Pavanadasivam, "Bioproduction of Trichothecenes from *Myrothecium Species*," 186th National Meeting of the ACS, Symposium on "Update on Antitumor Compounds from Fermentations," Washington, D.C., August 28-September 2, 1983.
4. B. B. Jarvis and S. L. Greene, "Analysis for Macrocyclic Trichothecenes," Eastern Analytical Symposium, New York, NY, November 16, 1983.
5. B. B. Jarvis, "Bioproduction and Analysis of Macrocyclic Trichothecenes," FASEB Summer Research Conference, Saxtons River, VT, June 25, 1984.

000370

6. B. B. Jarvis, "Preparative Liquid Chromatography in Natural Products Chemistry," Preparative Liquid Chromatography Symposium, Washington, D.C., May 15, 1985.
7. B. B. Jarvis, "Chemistry and Biology of the Trichothecene Mycotoxins," S. J. Cristol Symposium, Boulder, Colorado, May 30, 1985.
8. B. B. Jarvis, "Molecular Basis of Action for the Trichothecenes," FASEB Summer Research Conference, Copper Mountain, CO, July 9, 1986.
9. B. B. Jarvis, "New and Potent Macrocyclic Trichothecenes," 15th IUPAC International Symposium on the Chemistry of Natural Products, August 19, 1986, The Hague, The Netherlands.
10. B. B. Jarvis, "Applications of Preparative Liquid Chromatography to Natural Products," 3rd Washington Symposium on Preparative Liquid Chromatography, May 5, 1987.
11. B. B. Jarvis, "Antibiotics from Higher Plants," X Annual Meeting on Micromolecular Evolution, Systematics and Ecology, May 3, 1988, Joao Pessoa, Brazil.
12. B. B. Jarvis, "Trichothecenes Immunoconjugates as Anticancer Agents," 29th Annual University of Buffalo Medicinal Chemistry Symposium, Buffalo, NY, June 3, 1988.
13. B. B. Jarvis, "Mycotoxins - An Overview," 9th World Congress on Animal, Plants, and Microbial Toxins, Stillwater, OK, August 1, 1988.
14. B. B. Jarvis, "Trichothecenes in *Baccharis* Species," 7th International IUPAC Symposium on Mycotoxins and Phycotoxins, Tokyo, Japan, August 19, 1988.
15. B. B. Jarvis, "Phytotoxicity of Macrocyclic Trichothecenes Toward *Baccharis* Cell Lines," 5th International Congress of Plant Pathology, Kyoto, Japan, August 2, 1988.
16. B. B. Jarvis, "Fungal Toxins Present in Female *Baccharis* Plants: A Case of Horizontal Gene Transfer?," 6th Annual Meeting of the Eastern Branch of the Entomological Society of America, Syracuse, NY, October 3, 1988.
17. B. B. Jarvis, "Mycotoxins - An Overview," 9th World Congress on Animal, plant, and Microbial Toxins, Stillwater, OK, August 1, 1988.
18. B. B. Jarvis, G. A. Bean, J. O. Midiwo, M. B. Aboul-Nasr, J. Kuti, and N. Mokhtari, "The Mystery of Trichothecenes in Higher Plants," 7th International IUPAC Symposium on Mycotoxins and Phycotoxins, Tokyo, Japan, August 17, 1988.
19. B. B. Jarvis, J. Kuti, and G. A. Bean, "Phytotoxicity of Macrocyclic Trichothecenes Toward *Baccharis* Cell Lines," 6th International Congress on Plant Pathology, Kyoto, Japan, August, 22, 1988.

20. B. B. Jarvis, "Mycotoxins in Brazilian Higher Plants," 16th International Symposium on the Chemistry of Natural Products, Monterrey, Mexico, April, 28, 1989.
21. B. B. Jarvis, "Mycotoxins and Indoor Air Quality," Symposium on Biological Contaminants in Indoor Environments, AST STP 1071, Boulder, CO., June, 28, 1989.
22. W. G. Sorenson and B. B. Jarvis, "Mycotoxins in Airborne Spores of *Stachybotrys atra*," 8th International Bioterrorism and Biodegradation Symposium, Windsor, Canada, August 27, 1990.
23. B. B. Jarvis, "Isolation and Characterization of Trichothecenes from Plant and Microbial Sources," 43rd Southeastern ACS Meeting, Richmond, VA, Nov. 14, 1991.
24. B. B. Jarvis, "Role of Secondary Metabolites in Evolution," Inaugural Conference of the Kenyan Chemical Society, Nairobi, Kenya, June 7, 1993.
25. B. B. Jarvis, S. C. Trapp, and T M. Hohn, "Fungal Toxins Produced by Brazilian *Baccharis* Species: The Case for Horizontal Gene Transfer," Compositae Systematics Biology Utilization International Conference, KEW Gardens, Kew, England, August 2, 1994.
26. B. B. Jarvis, "Toxigenic Fungi in Indoor Air," Fungi and Bacteria in Indoor Air Environments, Saratoga, NY, Oct. 6, 1994.
27. B. B. Jarvis, "Secondary Metabolites as a Driving Force in Evolution," 17th Annual Meeting on Micromolecular Evolution, Systematics, and Ecology, Rio de Janeiro, Brazil, April 28, 1995.
28. B. B. Jarvis, "Toxigenic Fungi in the Air We Breathe," 5th Pan American Symposium on Animal, Plant, and Microbial Toxins, Frederick, MD, August 2, 1995.
29. B. B. Jarvis and J. D. Miller, "Secondary Metabolism, Complexity, and Evolution," Annual Meeting of the Phytochemical Society of North America, Sault Ste. Marie, Ontario, Canada, August 13, 1995.
30. B. B. Jarvis, "Chemical Analyses of *S. atra* Isolates from Cleveland Homes," Indoor Air and Diseases in Cleveland Infants, a workshop held at the CDC, Atlanta, GA, August 25, 1995.
31. B. B. Jarvis, "Analysis of Building Materials for Mycotoxins," AOAC International Annual Meeting, Nashville, TN, September 20, 1995.
32. B. B. Jarvis, "Anticancer Agents: Past, Present, and Future," Cellular & Molecular Biology 2nd World Congress, Ottawa, CA, September 6, 1996.
33. B. B. Jarvis, "*Stachybotrys* in the Air," Gordon Research Conference, Henniker, NH, June 16, 1997.

000372

34. B. B. Jarvis and S. Hinkley, "*Stachybotrys Toxins*," 3rd International Conference on Bioaerosols, Fungi and Mycotoxins, Saratoga, NY, Sept. 23-25, 1998.
35. B. B. Jarvis, "Fungal Toxins in the Environment," NSF Workshop on Natural Products Chemistry, Cairo, Egypt, Feb. 20-26, 1999.
36. B. B. Jarvis, "Novel Toxins from *Stachybotrys* and *Memnoniella* Species," Gordon Research Conference, Henniker, NH, June 22, 1999.
37. B. B. Jarvis, "Mycotoxins in the Indoor Air: Case Studies of *Stachybotrys chartarum*," Moulds in Buildings Symposium, Rungstedgaard, Denmark, October 9, 1999.
38. B. B. Jarvis, "Genomic Structure of Natural Product Biosynthetic Pathways," 3rd Annual Copenhagen Natural Product Symposium, Dec. 10, 1999.
39. B. B. Jarvis, "Natural Toxins in the Air We Breathe and the Food We Eat," Joint Workshop in Life Science, Universities of Maryland and Tel Aviv, Jan. 11, 2000.

C. Other Creative and Scholarly Activities

1. Patents: S. M. Kupchan, B. B. Jarvis, and R. G. Dailey, Jr., "Novel Antileukemic Trichothecene Epoxides," August 14, 1979, U.S. Patent No. 4,164,584.

D. Instruction (since 1986)

1. Courses taught

<u>Semester</u>	<u>Course</u>	<u>No. of Students</u>	<u>Hours</u>
Spring 1986	CHEM 648A	20	2
	CHEM 648C	12	1
Fall 1986	CHEM 235	30	4
Spring 1987	CHEM 648A	22	2
	CHEM 648C	18	1
Fall 1987	On leave at National Science Foundation		
Spring 1988	On leave at National Science Foundation		
Fall 1988	CHEM 233	250	4
	CHEM 648B	15	1
Spring 1989	CHEM 233	150	4
Fall 1989	CHEM 233	120	4
Spring 1990	CHEM 243	280	4
	CHEM 648 (team taught graduate class in natural products)		
Fall 1990	CHEM 235	15	4
Spring 1991	CHEM 491	6	3
	CHEM 898D (Org. Seminar)	30	1
Fall 1991	CHEM 233H	48	4

[REDACTED]

E. Extension Work -none

F. Service

1. (a) Member of: American Chemical Society (ACS); American Association for the Advancement of Science (AAAS); Sigma Xi; and Amer. Soc. of Pharmacognosy.
- (b) Member of the ad hoc review committee for NIH-NCI for contracts dealing with isolation of anticancer compounds from plants and fermentations (10/20/83; 10/5/84; 10/11/85).
- (c) Member of NIH-NCI Developmental Therapeutics Contracts Review Committee (7/1/83-6/30/87).
- (d) Member of the ad hoc NIH Study Section Medicinal Chemistry Section B (12/16/86)
- (e) Member of NIH-NCI Aids Contract Review Panels, (9/22/87 and 10/19/87).
- (f) Editorial Board of *Mycotoxin Research*.
- (g) Program Manager (Organic Synthesis), National Science Foundation (8/87-8/88).
- (h) Member of *ad hoc* review committee for AID program in Science and Technology Cooperation (10/88).
- (i) Consultant member to NIH-NCI Developmental Therapeutics Contracts Review Committee (12/12/89)
- (j) Organizer of NSF-sponsored Workshop in Natural Products Chemistry held 5/31/90 - 6/1/90 in Sao Paulo, Brazil.
- (l) Member of the Committee on Bioaerosols for the American Conference of governmental Hygienists (1990-1995).
- (m) Member of the *ad hoc* NIH-NCI Developmental Therapeutics Contracts Review Committee (1/16-18/91).

- (n) Member of the NIH-RCMI site visit review committee, Univ. of Puerto Rico (5/19-22/91).
 - (o) Member of the *ad hoc* NIH Study Section Medicinal Chemistry Section B (1/7/91).
 - (p) Member of *ad hoc* review committee for AID program in Science and Technology Cooperation (10/16/91).
 - (q) Member of the *ad hoc* NIH-DABR Study Section (10/13-15//92).
 - (r) Member of Expert Panel on Medical and Environmental Management of *Stachybotrys atra* Contamination (New York City, 5/7/93).
 - (s) Chair, NIH-NCI Developmental Therapeutics Contracts Review Committee for "National Cooperative Natural Products Drug Discovery Groups," (3/8-10, 1995).
 - (t) Member of the NIH Study Section for Multidisciplinary Special Emphasis Panel, SBIR (7/24-25/95).
 - (u) Member of the NSF Panel for Instrumentation for Undergraduate Science (1/17-20/96)
 - (v) Finalist Judge, Westinghouse Talent Search, Washington D.C. (3/14-16/96)
 - (w) Member, NIH-NCI Developmental Therapeutics Contracts Review Committee for "National Cooperative Natural Products Drug Discovery Groups," (6/17-19, 1996; 12/12-14/97).
2. (a) Department: Chairman of Organic Division (1984-87); Associate Chairman (1988-89; 1992-1993; 1998-1999); Acting Chairman (1989-90); Chairman (1993-1998). Committees: Member of (1) Faculty and Graduate Student Awards (1983-86; Chairman 1985-86); (2) Faculty Advisory Committee (1984-87); (3) Ad hoc Search Committee (1984-86; Chairman 1985-86); (4) Analytical Service Committee (1983-present; Chairman 1983-85); (5) Chairman of the Library/Colloquium Committee (1985-86); (7) Graduate Admissions (1988-89); (8) Chair Executive Search Committee (1989-90); (9) Chair, FAC (1990-91); (10) Chair, Library and Colloquium Committee (1990-92); (11) FAC (1991-1993); Co-Chair Organic-Biochemistry Faculty Search (1993).
- (b) University: (1) Faculty Senate (1982-84); (2) ALSC PCC Committee (1984-87); (3) Library Advisory Committee (1986-87); (4) Member of Search Committee for Associate Dean for Undergraduate Affairs, College of Agricultural and Life Sciences (1988-89); (5) Graduate Council Committee on Fellowships (1989-92); (6) Provost's Limited Enrollment Committee (1990-92); (7) Chair, Search Committee for Associate Dean for Undergraduate Studies for the Colleges of Agriculture and Life Sciences (1990); (8) Provost's Review Committee on

000376

Microbiology (1991); (9) Research Award Committee for Colleges of Agriculture and Life Sciences (1991); (10) Packard Award Selection Committee (1991); (11) Pelzar Dissertation Award Committee (1991); (12) Faculty Senate (1991-93); (13) Chair, University Graduate Fellowship Committee (1991-92); (14) Provost Search Committee (1992-93); (15) Senate Faculty Affairs Committee (1992-93); Chair, Botany Chair Search Committee (1993-94; 1994-95); Key Scholar Selection Committee (1994); Campus Senate Faculty Affairs Committee; Search Committee for Director of ORAA (1994-95) & since 1996, committees too numerous to remember.

G. International Activities

Spoke at a symposia in Australia, and Japan (see Papers Presented at Professional Meetings), gave seminars in Hungary, China, Poland, Egypt, and Japan (see Invited Seminars), invited participant in 6th IUPAC Symposium on Mycotoxins and Phycotoxins, Pretoria, South Africa, July 22-25, 1985), and 15th IUPAC, The Hague, Netherlands, August 17-22, 1986. Invited to speak at International Symposia in Brazil (May 1988), Buffalo, NY (June 1988), Stillwater, OK (July 1988), Japan (August 1988), and Mexico (April 1989) and Kenya (June 1993), organizer of NSF-sponsored workshop in natural products held in Brazil (5/90). Consultant for United Nations project on Production and Use of Silicas in (Hanoi, Vietnam, 6/15-30/90). Visited and spoke at Research Institutes and Universities in Sri Lanka (1975 and 1980), Egypt (1987 and 1991), and Kenya (1993), Kingston University, Kingston-on-the-Thames, England (1994). Workshop in Natural Products Participant, Cairo, Egypt, (1999).

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000378

MICHAEL W. PARIZA, PH.D. is director of the Food Research Institute (FRI), Wisconsin Distinguished Professor, and chair of the Department of Food Microbiology and Toxicology, University of Wisconsin--Madison. He holds affiliate appointments in the Wisconsin Clinical Cancer Center, the Environmental Toxicology Center, and the departments of Nutritional Sciences and Food Science at the University of Wisconsin--Madison. Dr. Pariza is a member of numerous scientific societies. He is a past-chairman of the Food Microbiology Division of the American Society for Microbiology, a past-member of the Institute of Medicine's Food Forum, and was a member of the National Academy of Sciences Committee on Comparative Toxicity of Naturally Occurring Carcinogens. He has been instrumental in organizing several national and international scientific conferences, has authored or co-authored over 130 articles and publications, and holds more than 25 U.S. patents. He is also a member of the Board of Trustees of the International Life Sciences Institute--North America and a Fellow of the Institute of Food Technologists. Dr. Pariza received his B.S. in Bacteriology at the University of Wisconsin--Madison and his M.S. and Ph.D. in Microbiology at Kansas State University. He then completed three years of postdoctoral study with Professor Van R. Potter at the McArdle Laboratory for Cancer Research at the University of Wisconsin--Madison and joined the faculty of the Food Research Institute, Department of Food Microbiology and Toxicology in 1976. Dr. Pariza's research focuses on conjugated linoleic acid, the biological activity of which was discovered in his laboratory.

MICHAEL W. PARIZA - CURRICULUM VITAE

Academic Rank: Director, Food Research Institute; Wisconsin Distinguished Professor and Chair, Department of Food Microbiology and Toxicology.

Education:

Ph.D.	Kansas State University	1973	Microbiology
M.Sc.	Kansas State University	1969	Microbiology
B.Sc.	University of Wisconsin-Madison	1967	Bacteriology

Professional Experience:

- Kansas State University: Graduate Teaching Assistant, Graduate Research Assistant, 1967-1969.
- U.S. Army (Armed Forces Institute of Pathology, Washington, D.C.): Biological Sciences Assistant, 1969-1971.
- Kansas State University: Graduate Teaching Assistant, Graduate Research Assistant, 1971-1973.
- The University of Wisconsin-Madison (McArdle Laboratory for Cancer Research): NIH Postdoctoral Traineeship with Professor Van R. Potter, 1973-1976.
- The University of Wisconsin-Madison (The Food Research Institute, Department of Food Microbiology and Toxicology): Assistant Professor, 1976-1981; Associate Professor, 1981-1984; Professor, 1984-Present; Associate Department Chair, 1981-1982; Department Chair, 1982-Present; Director, Food Research Institute, 1986-present.
- Wisconsin Distinguished Professor, 1993-present.
- Affiliate appointments at the University of Wisconsin: Wisconsin Comprehensive Cancer Center; Environmental Toxicology Center; Department of Nutritional Sciences; Department of Food Science.

Membership in Scientific Organizations:

The American Association for Cancer Research
The American Society for Microbiology
The American Association for the Advancement of Science
The Society of Sigma Xi
The Institute of Food Technologists
The Toxicology Forum
FASEB (The American Institute of Nutrition; The American Society for Clinical Nutrition)
The American Chemical Society
The American Oil Chemists' Society

Special Professional Recognition (1986-present):

- Co-Chair of Organizing Committee, Co-Editor of Proceedings, and Keynote Speaker for an international symposium entitled "Calories and Energy Expenditure in Carcinogenesis," held in Washington, DC, in 1986.
- Chair, Food Microbiology Division of the American Society for Microbiology, 1986/87.

- Member, Board of Trustees, International Life Sciences Institute - North America, 1986-present.
- Invited twice (1986 and 1989) to lecture at the annual American Cancer Society's Science Writers Symposium.
- Invited lecture at a symposium on Diet and Health, held in Vevey, Switzerland, in 1987; sponsored by the Nestle Company.
- Sabbatical at Colworth House in Great Britain, guest of the Unilever Company, summer, 1987.
- Chair, Committee on Diet and Health for CAST (the Council on Agricultural Science and Technology). Report issued in 1987.
- Invited lecture at an international conference on Anticarcinogens and Antimutagens in the Diet, held in Ohito, Japan, in 1988; also lectured at the Toyama Public Health Laboratory, and the Kikkoman Company.
- Author of Food, Diet, and Health Relationships for the Issues and Challenges Section, United States Department of Agriculture Five-Year Plan, 1988.
- Invited lecture at an international symposium on Diet and Cancer held at the Karolinska Institute, Stockholm, Sweden, in 1989.
- Invited lecture at a meeting on Biotechnology and Food Safety held at Cornell University, Ithaca, NY, in 1989.
- Invited lecture at an international symposium on Anticarcinogens in the Diet, held in Charleston, SC, in 1989.
- Member, Document Drafting Committee, International Food Biotechnology Council, 1989/90.
- Invited lecture at an international symposium on Food Biotechnology sponsored by the International Food Biotechnology Council, held in Washington, DC, in 1989.
- Chair of Organizing Committee and speaker at an international symposium, Mutagens and Carcinogens in the Diet, held in Madison, WI, in 1989.
- Member, Scientific Advisory Committee for Universal Foods Corporation, 1989 to present.
- Invited lecture on anticarcinogen research in two separate symposia at the 1990 annual FASEB meeting.
- Invited lecture at an international conference on food safety at Michigan State University, in 1990.
- Invited lecture on risk assessment at an EPA-sponsored symposia on Pesticidal Transgenic Plants, held in Annapolis, MD, in 1990.
- Advisor on toxicology matters to the Madison Metropolitan Sewerage District Commission, 1990 to present.
- Invited lecture at a symposium on Anticarcinogens, sponsored by the National Cancer Institute and held in San Diego, CA, in 1991.
- Invited lecture at an international symposium on cancer prevention organized by former Surgeon General C. Everett Koop, held in Washington, DC, in 1991.
- Keynote speaker at the annual convention of the National Association of State Departments of Agriculture, held in Las Vegas, NV, in 1991.
- Invited lecture on risk assessment at symposium on "Industrial Ecology," sponsored by the National Academy of Sciences and held in Washington, DC, in 1991.
- Invited lecture at the 5th International Congress on Oxygen Radicals, held at Kyoto, Japan, in 1991.

- Breakfast speaker at mid-year meeting of the National Association of State Departments of Agriculture, held in Washington, DC, in 1992.
- Invited lecture on natural toxicants at symposium on plant Biotechnology sponsored by The Institute of Medicine and held in Irvine, CA, in 1992.
- Invited lecture at symposium on anticarcinogens from dairy products, IFT Annual Meeting, 1992.
- Invited lecture at symposium on anticarcinogens, American Chemical Society Annual Meeting, 1992.
- Member, 1993 and 1998 Program Planning Committees, American Association for Cancer Research.
- Keynote speaker at the 1993 annual meeting of the Weed Science Society of America.
- Member, Institute of Medicine's Food Forum, 1993-present.
- Member, National Academy of Sciences Committee on Comparative Toxicity of Naturally Occurring Carcinogens, 1993-1996.
- Invited participant at the U.S. Congressional staff briefing conducted by the Environmental and Energy Study Conference on the safety and regulation of genetically engineered foods, 1993.
- Presented two invited lectures at the Health Protection Branch, Ottawa, Canada, November 1993.
- Lecturer and session chair for two food safety symposia sponsored by the Ceres Forum of Georgetown University, Fall 1993.
- Invited participant at the National Yogurt Association International Conference entitled "Yogurt: Myth Versus Reality," held on October 26-27, 1993 in Washington, DC.
- Keynote lecturer and session chair for symposium on food safety, 1994 annual convention of the American Association for the Advancement of Science.
- Invited participant for two meetings of the U.S.-Japan Cooperative Cancer Research Program, held in Honolulu in March 1994.
- Invited participant (lecture and session chair) at the 1994 summer Toxicology Forum meeting.
- Invited lecture entitled "The Effect of Diet on National Health Care Costs" at the National Planning Association's Food and Agriculture Committee meeting of September 9, 1994 held in Washington, D.C.
- Invited lecture entitled "A Review of Foodborne Pathogens and Risk" at the annual meeting of the American Dietetic Association in a symposium entitled "Meat and Poultry Safety: From Farm to Table," held on October 18, 1994 in Orlando, FL.
- Invited panelist at a session entitled "Interrelationships of Food, Diet, and National Health" at the annual meeting of the National Association of State Universities and Land-Grant Colleges held on November 6, 1994 in Chicago, IL.
- Invited participant in a conference entitled "National Forum on Meeting the Challenge: Health, Safety, and Food for America" sponsored by the White House Office of Science and Technology Policy held on November 21-22, 1994 in Washington, DC.
- Invited lecture entitled "Foodborne Microbial Pathogens" at the International Food and Lifestyles Media Conference, January 30, 1995, Las Vegas, NV.
- Co-organizer of and participant in a U.S.-Japan Cooperative Cancer Research Program, March, 1995, Maui, HI.

- Testified at a hearing on the FDA and the future of the American biomedical and food industries, held by the U.S. Senate Committee on Labor and Human Resources, chaired by Senator Nancy Kassebaum, April 5, 1995, Washington, DC.
- Invited participant in an IFT Basic Symposium on lipids and health, June 2-3, 1995, Anaheim, CA.
- Discussed reform of the Delaney Clause at a press conference on reforming the federal regulatory process held at the Senate Office Building, July 12, 1995, Washington, DC.
- Invited participant at the Third International Symposium on "Infant Nutrition in the Prevention of Chronic Pathology," held on September 20, 1995, in Alicante, Spain.
- Invited to lecture at FDA on "Conjugated linoleic acid: A newly recognized nutrient," October 4, 1995, Washington, DC.
- Three invited lectures on "Functional foods and research on conjugated linoleic acid (CLA)," December, 1995, in Japan (International Conference on Food Factors that Prevent Cancer in Hamamatsu City; Kikkoman Corporation in Noda City; Japanese National Cancer Institute in Tokyo).
- Testified at a hearing on "The Need for FDA Reform" held by the Subcommittee on Health and Environment, Committee on Commerce, U.S. House of Representatives, Washington, D.C., February 27, 1996.
- Invited participant (presented 2 lectures) at a symposium on conjugated linoleic acid sponsored by the American Oil Chemists Society, April 29, 1996, in Indianapolis.
- Invited participant and session moderator at a Workshop on Individual Fatty Acids and Cancer sponsored by the International Life Sciences Institute-North America, June 4-5, 1996, Washington, D.C.
- Recipient of 1996 Marqueta C. Huyck Endowed Lectureship, Wayne State University, Detroit, MI. Lecture title, "CLA: The fat that reduces obesity."
- Invited participant at an international symposium, "Fundamentals of Cancer Prevention," sponsored by the Princess Takamatsu Cancer Research Fund, Tokyo, Japan, November, 1996.
- Speaker at February 1997 dinner meeting of the Chicago Section of the Institute of Food Technologists, Chicago, IL.
- Lecturer at 1997 symposium series, "Modern Views in Nutrition," Iowa State University, Ames, IA.
- Chair of Symposium on "Enhancing the Regulatory Approval Process for Food Ingredient Technologies," sponsored by the National Academy of Sciences and the Institute of Medicine (Food and Nutrition Board, Food Forum), May 6-7, 1997.
- Elected Institute of Food Technologists Fellow, 1997.
- Invited lecture at 6th International Congress on Clinical Nutrition, at Banff, Alberta, Canada, July, 1997.
- Invited lecture at 16th International Congress of Nutrition, Montreal, Canada, July, 1997.
- Chair of Organizing Committee for the first CLA Forum, held at the University of Wisconsin-Madison, August, 1997 (highlights published in *INFORM* 9:69-73, 1998).
- Invited lecture at symposium sponsored by Best Foods, New Jersey, September, 1997.
- Invited lecture at Michigan State University, October, 1997.
- Invited lecture at the Japanese National Cancer Center, January, 1998.
- Invited lecture at the University of Illinois, February, 1998.

- Invited lecture, "Functional Foods: Technology, Functionality and Health Benefits," at Experimental Biology 98 symposium held in San Francisco, CA sponsored by ILSI (International Life Sciences Institute), April, 1998.
- Invited lecture on conjugated linoleic acid research for the Board of the Finnish Food Foundation, Helsinki, Finland, May, 1998.
- Two invited lectures at the Finnish Food Congress in Helsinki, Finland: "Food Safety: Risks and Management" (keynote address), and *E. coli* O157:H7 research at FRI, May, 1998.
- Invited lecture on conjugated linoleic acid at the National Cancer Institute, Bethesda, MD, May, 1998.
- Chair of session, "Modification of Cancer Risk by Inulin and Oligofructose," at conference entitled "Nutritional and Health Benefits of Inulin and Oligofructose" held at NIH (National Institute of Health) in Bethesda, MD, May, 1998.
- Invited lecture on conjugated linoleic acid at The Toxicology Forum, Aspen, CO, July, 1998.
- Invited lecture on conjugated linoleic acid at a symposium at Iowa State University, Ames, IA, September, 1998.
- Invited lecture on conjugated linoleic acid at a symposium sponsored by the Society of Toxicology, Reston, VA, October, 1998
- Co-organizer of the first "International Workshop on Conjugated Linoleic Acid Analysis," held in Washington, DC, January, 1999.
- Invited lecture on conjugated linoleic acid at the 1999 annual meeting of the American Oil Chemists Society.
- Invited lecture on conjugated linoleic acid at the 1999 annual meeting of the Institute of Food Technologists (IFT).
- Invited lecture on conjugated linoleic acid at the 40th International Conference on the Biochemistry of Lipids.
- Invited lecture on conjugated linoleic acid at the 1999 Steenbock Symposium.

Research Focus:

Conjugated linoleic acid

Patents:

1. Pariza, M.W., and Ha, Y.L. Methods of preventing oxidation, quenching singlet oxygen, and inhibiting mold growth and novel compositions thereof. U.S. 5,017,614
2. Pariza, M.W., and Ha, Y.L. Methods of chelating metal and novel compositions therefor. U.S. 5,070,104
3. Pariza, M.W., and Ha, Y.L. Octadecadienoic phospholipid esters, antioxidant and mold inhibiting compositions. U.S. 5,208,356
4. Cook, M.E., and Pariza, M.W. Methods for preventing weight loss, reduction in weight gain, and anorexia due to immune stimulation. U.S. 5,430,066

5. Cook, M.E. and Pariza, M.W. Method for increasing the efficiency of feed conversion in animals. U.S. 5,428,072
6. Cook, M.E., Pariza, M.W., Lee, K.N., and Wentworth, B.C. Method for controlling bird populations. U.S. 5,504,114.
7. Cook, M.E., Pariza, M.W., and Park Y. Method for reducing body fat in animals. U.S. patent application. U.S. 5,554,646.
8. Cook, M.E., Pariza, M.W., Yang, X., and Devoney, D. Methods of treating animals to maintain or increase CD-4 and CD-8 cell populations. U.S. 5,674,901.
9. Cook, M.E., and Pariza, M.W. Dietetic foods containing conjugated linoleic acids. U.S. 5,760,082.
10. Cook, M.E., Cook, E.B., Stahl, J.L., Graziano, F.M., and Pariza, M.W. Methods of attenuating the allergic response in animals. U.S. 5,585,400.
11. Cook, M.E. and Pariza, M.W. Methods of treating animals to maintain or enhance bone mineral content and compositions for use therein. U.S. 5,804,210.
12. Satter, L.D., Dhiman, T.R., and Pariza, M.W. Method of increasing the CLA content of cow's milk. U.S. 5,770,247.
13. Cook, M.E., Pariza, M.W., and Jerome, D.L. Use of CLA to reduce the incidence of valgus and vargus leg deformities in poultry. U.S. 5,760,083.
14. Pariza, M.W. and Yang, X. Method of producing conjugated fatty acids. U.S. 5,856,149.
15. Pariza, M.W. and Lee, K.N. Method to reduce secretion of apolipoprotein β . U.S. 5,837,733.
16. Cook, M.E., Pariza, M.W., Kim, S., and DeVoney, D. Methods of treating animals to enhance natural killer lymphocyte function. U.S. 5,914,346.
17. Cook, M.E., Park, Y., and Pariza, M.W. Method for controlling body fat and/or body weight in animals and pharmaceutical compositions for use therein. U.S. 5,855,917.
18. Cook, M.E., Jerome, D.L., Pariza, M.W., and Buege, D.R. Method for increasing fat firmness and improving meat quality in animals. U.S. 5,851,572.
19. Cook, M.E. and Pariza, M.W. Method for maintaining an existing level of body fat and/or body weight. U.S. 5,814,663.
20. Cook, M.E. Pariza, M.W., Yang, X., and Devoney, D. Methods of treating animals to maintain or increase CD-4 and CD-8 cell populations. U.S. 5,827,885.

000385

21. Cook, M.E., Jerome, D., and Pariza, M.W. Method for selectively altering body fat level, feed efficiently, or weight gain. U.S. 6,020,378.
22. Pariza, M.W. and Yang, X. Methods of treating animals to maintain or increase CD-4 and CD-8 cell populations. U.S. 6,020,376.
23. Pariza, M.W. and Yang, X. Method of producing conjugated fatty acids. U.S. 5,856,149.

-- (Note: Foreign patent equivalents on each of these have issued or are pending.) --

Publications:

1. Pariza, M. W. and Iandolo, J. J. 1969. Coagulase production by injured *Staphylococcus aureus* MF-31 during recovery. *Appl. Microbiol.* 17:836-838.
2. Rosenthal, L. J., Martin, S. E., Pariza, M. W. and Iandolo, J. J. 1972. Ribosome synthesis in thermally shocked cells of *Staphylococcus aureus*. *J. Bacteriol.* 109:243-249.
3. Pariza, M. W. and Iandolo, J. J. 1974. Base ratio and DNA homology studies on six *Staphylococcus aureus*. *Appl. Microbiol.* 27:317-323.
4. Pariza, M. W. and Iandolo, J. J. 1974. Determination of genome size of selected bacteriophages of *Staphylococcus aureus*. *Appl. Microbiol.* 28:510-512.
5. Pariza, M. W., Becker, J. E., Yager, J. D., Jr., Bonney, R. J. and Potter, V. R. 1974. Enzyme induction in primary cultures of rat liver parenchymal cells, pp. 267-284. In W. Nakahara, T. Ono, T. Sugimura and H. Sugano (eds.) *Differentiation and control of malignancy of tumor cells*. University of Tokyo Press, Tokyo, Japan.
6. Kletzien, R. F., Pariza, M. W., Becker, J. E. and Potter, V. R. 1975. A method using 3-O-methyl-D-glucose and phloretin for the determination of intracellular water space of cells in monolayer cultures. *Anal. Biochem.* 68:537-544.
7. Kletzien, R. F., Pariza, M. W., Becker, J. E. and Potter, V. R. 1975. A "permissive" effect of dexamethasone on the glucagon induction of amino acid transport in cultured hepatocytes. *Nature* 256:46-47.
8. Pariza, M. W., Yager, J. D., Jr., Goldfarb, S., Gurr, J. A., Yanagi, S., Grossman, S. H., Becker, J. E., Barber, R. A. and Potter, V. R. 1975. Biochemical, autoradiographic and electron microscopic studies on adult rat liver parenchymal cells in primary culture, pp. 137-167. In L. E. Gerschenson and E. B. Thompson (eds.) *Gene expression and carcinogenesis in cultured liver*. Academic Press, New York.

9. Yager, J. D., Jr., Pariza, M. W., Becker, J. E. and Potter, V. R. 1975. DNA synthesis in primary cultures of parenchymal cells isolated from regenerating rat liver, pp. 148-151. In R. Lesch and W. Reutter (eds.) *Liver regeneration after experimental injury*. Stratton Intercontinental Medical Book Corporation, New York.
10. Pariza, M. W., Yanagi, S., Gurr, J. A., Morris, H. P. and Potter, V. R. 1976. Ornithine decarboxylase activity and DNA synthesis in Morris hepatomas 5123-C and 7800. *Life Sci.* 18:39-48.
11. Kletzien, R. F., Pariza, M. W., Becker, J. E., Butcher, F. R. and Potter, V. R. 1976. Induction of amino acid transport in primary cultures of adult rat liver parenchymal cells by insulin. *J. Biol. Chem.* 251:3014-3020.
12. Pariza, M. W., Yanagi, S., Gurr, J. A., Morris, H. P. and Potter, V. R. 1976. Fasting does not abolish the diurnal oscillation of ornithine decarboxylase in Morris hepatoma 5123-C. *Life Sci.* 19:1553-1558.
13. Pariza, M. W., Butcher, F. R., Kletzien, R. F., Becker, J. E. and Potter, V. R. 1976. Induction and decay of glucagon-induced amino acid transport in primary cultures of adult rat liver cells: Paradoxical effects of cycloheximide and puromycin. *Proc. Natl. Acad. Sci. U.S.A.* 73:4511-4515.
14. Pariza, M. W., Kletzien, R. F., Butcher, F. R. and Potter, V. R. 1976. Inductions by hormones added singly, simultaneously or sequentially: What cultured hepatocytes can tell us about metabolic regulation in the whole animal. *Adv. Enzyme Regulation* 14:103-115.
15. Kletzien, R. F., Pariza, M. W., Becker, J. E. and Potter, V. R. 1976. Hormonal regulation of amino acid transport and gluconeogenesis in primary cultures of adult rat liver parenchymal cells. *J. Cell. Physiol.* 89:641-646.
16. Pariza, M. W., Butcher, F. R., Becker, J. E. and Potter, V. R. 1977. Cyclic AMP-independent induction of amino acid transport by epinephrine in primary cultures of adult rat liver cells. *Proc. Natl. Acad. Sci. U.S.A.* 74:234-237.
17. Pariza, M. W., Kletzien, R. F. and Potter, V. R. 1977. A model for the "permissive" effect of glucocorticoids on the glucagon induction of amino acid transport in cultured hepatocytes, pp. 379-388. In R. T. Acton and J. D. Lynn (eds.) *Proc. International Cell Culture Congress*. Academic Press, New York.
18. Goldfarb, S., Barber, T. A., Pariza, M. W. and Pugh, T. W. 1978. Lipid synthesis and ultrastructure of adult rat hepatocytes during their first twenty-four hours in culture. *Exptl. Cell Res.* 117:39-46.
19. Giger, O. and Pariza, M. W. 1978. Depression of amino acid transport in cultured rat hepatocytes by purified enterotoxin from *Clostridium perfringens*. *Biochem. Biophys. Res. Commun.* 82:378-383.

20. Saccone, G. T. P. and Pariza, M. W. 1978. Effects of dietary butylated hydroxytoluene and phenobarbital on the activities of ornithine decarboxylase and thymidine kinase in rat liver and lung. *Cancer Lett.* 5:145-152.
21. Pariza, M. W., Ashoor, S. H., Chu, F. S. and Lund, D. B. 1979. Effects of temperature and time on mutagen formation in pan-fried hamburger. *Cancer Lett.* 7:63-69.
22. Pariza, M. W., Ashoor, S. H. and Chu, F. S. 1979. Mutagens in heat-processed meat, bakery and cereal products. *Food Cosmet. Toxicol.* 17:429-430.
23. Pariza, M. W. 1979. Food safety: from the eye of a hurricane. *Professional Nutritionist* 11:11-14 (commissioned article).
24. Giger, O. and Pariza, M. W. 1980. Mechanism of action of *Clostridium perfringens* enterotoxin: effects on membrane permeability and amino acid transport in primary culture of adult rat hepatocytes. *Biochem. Biophys. Acta* 595:264-276.
25. Ashoor, S. H., Dietrich, R. A., Chu, F. S. and Pariza, M. W. 1980. Proline enhances mutagen formation in ground beef during frying. *Life Sci.* 26:1801-1805.
26. Gayda, D. P. and Pariza, M. W. 1980. Rat hepatocytes in primary monolayer culture: a tool for investigating the regulation of carcinogen metabolism, pp. 1165-1168. In M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette and P. J. O'Brien (eds.) *Microsomes, drug oxidations and chemical carcinogenesis, Vol. II.* Academic Press, New York.
27. Saccone, G. T. P. and Pariza, M. W. 1981. Enhancement of hepatic microsome-mediated bacterial mutagenesis by the rat liver soluble protein fraction. *Mutat. Res.* 88:135-145.
28. Gayda, D. P. and Pariza, M. W. 1981. Activation of aflatoxin B₁ by primary cultures of adult rat hepatocytes: effects of hepatocyte density. *Chem. Biol. Interactions* 35:255-265.
29. Saccone, G. T. P., DasGupta, B. R. and Pariza, M. W. 1981. Enhancement of N-hydroxy-2-aminofluorene bacterial mutagenicity by the soluble protein fraction from rat liver and partial purification of the enhancement activity. *Cancer Res.* 41:4600-4605.
30. Pariza, M. W. 1982. Mutagens in heated foods. *Food Technol.* 36:53-56.
31. DasGupta, B. R. and Pariza, M. W. 1982. Purification of two *Clostridium perfringens* enterotoxin-like proteins and their effects on membrane permeability in primary cultures of adult rat hepatocytes. *Infect. Immun.* 38:592-597.
32. Pariza, M. W. (Consulting Reviewer). 1982. *Oncology Overview* on mutagens and carcinogens in cooked, smoked and charred foods. National Technical Information Service, Springfield, VA. Document #PB82-922914.

33. Hargraves, W. A., Dietrich, R. A. and Pariza, M. W. 1982. A new chromatographic method for separating mutagens from commercial beef extract and fried ground beef. In H. F. Stich (ed.) *Carcinogens and Mutagens in the Environment*, Vol. I, Food Products. CRC Press, Inc., pp. 223-229.
34. Hargraves, W. A. and Pariza, M. W. 1983. Purification and mass spectral characterization of bacterial mutagens from commercial beef extract. *Cancer Res.* 43:1467-1472.
35. Gayda, D. P. and Pariza, M. W. 1983. Activation of 2-amino-3-methylimidazo [4,5-f]quinoline and 2-aminofluorene for bacterial mutagenesis by primary monolayer cultures of adult rat hepatocytes. *Mutat. Res.* 118:7-14.
36. Gayda, D. P. and Pariza, M. W. 1983. Effects of carcinogens on hormonal regulation of gene expression in primary cultures of adult rat hepatocytes. *Carcinogenesis* 4:1127-1131.
37. Schwartz, S. J., von Elbe, J. H., Pariza, M. W., Goldsworthy, T. J. and Pitot, H. C. 1983. Inability of red beet betalain pigments to initiate or promote hepatocarcinogenesis. *Fd. Chem. Toxic.* 21:531-535.
38. Pariza, M. W. 1983. Carcinogenicity/toxicity testing and the safety of foods. *Food Technol.* 37(1):84-86.
39. Pariza, M. W., Loretz, L. J., Storkson, J. M. and Holland, N. C. 1983. Mutagens and modulators of mutagenesis in fried ground beef. *Cancer Res.* 43:2444s-2446s.
40. Pariza, M. W. and Foster, E. M. 1983. Determining the safety of enzymes used in food processing. *J. Food Prot.* 46:453-468.
41. Pariza, M. W. 1984. A perspective on diet, nutrition, and cancer. *J. Amer. Med. Assoc.* 251:1455-1458 (commissioned article).
42. Loretz, L. J. and Pariza, M. W. 1984. Effect of glutathione levels, sulfate levels and metabolic inhibitors on covalent binding of 2-amino-3-methylimidazo[4,5-f] quinoline and 2-acetylaminofluorene to cell macromolecules in primary monolayer cultures of adult rat hepatocytes. *Carcinogenesis* 5:895-899.
43. Hargraves, W. A. and Pariza, M. W. 1984. Mutagens in cooked foods. *J. Environ. Sci. Health C2(1):1-49.*
44. Pariza, M. W. and Hargraves, W. A. 1985. A beef-derived mutagenesis modulator inhibits initiation of mouse epidermal tumors by 7,12-dimethylbenz[a]anthracene. *Carcinogenesis* 6:591-593.
45. Pariza, M. W. 1985. Diet and cancer. American Council on Science and Health, New York, NY.

46. Pariza, M. W., Hargraves, W. A., Benjamin, H., Cristou, M., Jefcoate, C. R., Storkson, J., Albright, K., Kraus, D., Sharp, P., Boissonneault, G. A. and Elson, C. E. 1986. Modulation of carcinogenesis by dietary factors: I. Inhibition of carcinogenesis by a beef-derived mutagenesis modulator; and II. Role of net energy in enhancement of carcinogenesis by dietary fat. *Environ. Health Perspectives* 67:25-29.
47. Pariza, M. W., Hargraves, W. A. and Boissonneault, G. A. 1986. Modulation of carcinogenesis by a beef-derived mutagenesis modulator, and by dietary fat, in *Genetic Toxicology of the Diet*, I. Knudsen (ed.), Alan R. Liss, Inc., pp. 265-271.
48. Boissonneault, G. A., Elson, C. E. and Pariza, M. W. 1986. Net energy effects of dietary fat on chemically-induced mammary carcinogenesis in F344 rats. *JNCI* 76:335-338.
49. Pariza, M. W. 1986. Analyzing current recommendations on diet, nutrition and cancer. *Fd. Nutrit. News* 58:1-3 (commissioned article).
50. Pariza, M. W. 1986. Calories and energy expenditure in carcinogenesis. *Contemporary Nutrit.* Vol. XI, No. 4 (commissioned article).
51. Pariza, M. W. 1986. Calorie restriction, ad libitum feeding, and cancer. FASEB Symposium on Nutrition and Cancer. *Proc. Soc. Exptl. Biol. Med.* 183:293-298.
52. Pariza, M. W. 1986. Diet and cancer: science vs policy. *Pediatric Basics* 44:10-15.
53. Pariza, M. W. 1986. Symposium on calories and energy expenditure in carcinogenesis--executive summary. *Nutrit. Today*, July/August, 21-23.
54. Poirier, L. A., P. M. Newberne, and M. W. Pariza, (eds.). 1987. *Role of Essential Nutrients in Carcinogenesis*, Plenum Press, New York, 562 pp.
55. Boissonneault, G. A., Elson, C. E. and Pariza, M. W. 1987. Dietary fat and neoplasia: The role of net energy in enhancement of carcinogenesis; Effects of fat and calories on the immune system, In: *Role of Essential Nutrients in Carcinogenesis*, L. Poirier, P. Newberne and M. Pariza (eds.), Plenum Press, New York, pp. 85-98.
56. Pariza, M. W. (Task Force Chair). 1987. Diet and health. Council for Agricultural Science and Technology, Ames, Iowa.
57. Pariza, M. W. and Boutwell, R. K. 1987. Calories and energy expenditure in carcinogenesis: Historical Perspective. *Amer. J. Clin. Nutrit.* (supplement) 45:151-156.
58. Pariza, M. W. 1987. Fat, calories, and mammary carcinogenesis. *Amer. J. Clin. Nutrit.* (supplement) 45:261-263.
59. Pariza, M. W. 1987. Dietary fat, calorie restriction, *ad libitum* feeding, and cancer risk. *Nutrit. Rev.* 45:1-7 (commissioned lead article).

60. Ha, Y. L., Grimm, N. K. and Pariza, M. W. 1987. Anticarcinogens from fried ground beef: Heat-altered derivatives of linoleic acid. *Carcinogenesis* 8:1881-1887.
61. Pariza, M. W. 1988. Effects of calorie intake and expenditure on carcinogenesis, In: *Horticulture and Human Health. Contributions of Fruits and Vegetables*. Proc. 1st Int. Symp. Horticulture and Human Health. B. Quebedeaux and F. A. Bliss (eds.), Prentice-Hall, Englewood Cliffs, N.J., pp. 144-149.
62. Pariza, M. W. 1988. Dietary fat and cancer risk: Evidence and research needs. *Ann. Rev. Nutr.* 8:167-183.
63. Benjamin, H., Storkson, J., Tallas, P. G., and Pariza, M. W. 1988. Reduction of benzo(a)pyrene-induced forestomach neoplasms in mice given nitrite and dietary soy sauce. *Fd. Chem. Toxicol.* 26:671-678.
64. Ha, Y. L., Grimm, N. K., and Pariza, M. W. 1989. Newly recognized anticarcinogenic fatty acids: Identification and quantification in natural and processed cheeses. *J. Ag. Fd. Chem.* 37:75-81.
65. Johnson, E. A. and M. W. Pariza. 1989. Microbiological principles for the safety of foods, In: *International Food Regulation Handbook. Policy, Science, Law*. R. D. Middlekauff and P. Shubik (eds.), Marcel Dekker, Inc., New York, pp. 135-174.
66. Pariza, M. W. 1989. A perspective on diet and cancer, In: *Food Toxicology. A Perspective on Relative Risks*. S. L. Taylor and R. A. Scanlan (eds.), Marcel Dekker, Inc., New York, pp. 1-10.
67. Ha, Y. L., Storkson, J. and Pariza, M. W. 1990. Inhibition of benzo(α)pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. *Cancer Res.* 50:1097-1101.
68. Pariza, M. W. and Ha, Y. L. 1990. Newly recognized anticarcinogenic fatty acids, In: *Antimutagenesis and Anticarcinogenesis Mechanisms II*. Y. Kuroda, D. M. Shankel and M. D. Waters (eds.), Plenum Press, New York, pp. 167-170.
69. Pariza, M. W., H.-U. Aeschbacher, J. S. Felton, and S. Sato (eds). 1990. *Mutagens and Carcinogens in the Diet*, Wiley-Liss, Inc., New York, 332 pp.
70. Pariza, M. W. and Ha, Y. L. 1990. Conjugated dienoic derivatives of linoleic acid: Mechanism of anticarcinogenic effect, In: *Mutagens and Carcinogens in the Diet*. M. W. Pariza, H.-U. Aeschbacher, J. S. Felton, and S. Sato (eds.), Wiley-Liss, Inc., New York, pp. 217-221.
71. Pariza, M. W., and Ha, Y. L. 1990. Conjugated dienoic derivatives of linoleic acid: a new class of anticarcinogens. *Medical Oncology Tumor Pharmacology.* 7:169-172.

72. Pariza, M. W. 1990. Diet and cancer, In: *Foodborne Diseases*. D. O. Cliver (ed.), Academic Press, Inc., New York, New York, pp. 308-317.
73. Pariza, M. W. 1990. Perspectives on food safety and biotechnology, In: *Biotechnology and Food Safety*, Proceedings of the Second International Symposium. D. D. Bills and S. D. Kung (eds.), Butterworth-Heinemann, Boston, pp. 47-52.
74. Ha, Y. L., and Pariza, M. W. 1990. Anticarcinogenic conjugated dienoic derivatives of linoleic acid found in grilled ground beef: Isolation, identification, and mechanism of action, In: *The First Korean Conference on Science and Technology*. The Korean Federation and Science Association, Seoul, Korea, pp. 442-445.
75. Pariza, M. W. 1990. Evaluating the relative safety of biotechnologically produced foods. In: *Agricultural Biotechnology, Food Safety and Nutritional Quality for the Consumer*, NABC Report 2. Union Press of Binghamton, New York, pp. 167-173.
76. Benjamin, H., Storkson, J., Nagahara, A. and Pariza, M. W. 1991. Inhibition of benzo[a]pyrene-induced mouse forestomach neoplasia by dietary soy sauce. *Cancer Res.* 51:2940-2942.
77. Pariza, M. W. 1991. CLA, a new cancer inhibitor in dairy products. *Bull. Int. Dairy Fed.* 257:29-30.
78. Pariza, M. W. and Ha, Y. 1991. Fatty acids that inhibit cancer: Conjugated dienoic derivatives of linoleic acid. In: *Nutrients and Cancer Prevention*, K. N. Prasad and F. L. Meyskens, Jr. (eds.), Humana Press, Clifton, NJ, pp. 113-117.
79. Pariza, M. W., Ha, Y. L., Benjamin, H., Sword, J. T., Grüter, A., Chin, S. F., Storkson, J., Faith, N., and Albright, K. 1991. Formation and action of anticarcinogenic fatty acids. In: *Nutritional and Toxicological Consequences of Food Processing*, M. Friedman (ed.), Plenum Publishing Corp., New York, New York, pp. 269-272.
80. Pariza, M. W. 1991. Human health concerns associated with pesticidal transgenic plants. In: *Pesticidal Transgenic Plants: Product Development, Risk Assessment, and Data Needs*. U. S. EPA Conference Proceedings. Document #EPA/21T-1024, pp. 107-111.
81. Ip, C., Chin, S. F., Scimeca, J. A., and Pariza, M. W. 1991. Mammary cancer prevention by conjugated dienoic derivatives of linoleic acid. *Cancer Res.* 51:6118-6124.
82. Ha, Y. L. and Pariza, M. W. 1991. Naturally-occurring novel anticarcinogens: Conjugated dienoic derivatives of linoleic acid (CLA). *J. Korean Soc. Food Nutr.* 20(4):401-407.
83. Pariza, M. W. 1992. A new approach to evaluating carcinogenic risk. *Proc. Natl. Acad. Sci. U.S.A.* 89:860-861.
84. Pariza, M. W. 1992. Risk assessment. *Crit. Rev. Food Sci. Nutr.* 31:205-209.

85. Pariza, M. W. 1992. Chapter 4: Risk-benefit perceptions. In: *Food Safety Assessment*, J. W. Finley, S. F. Robinson, and D. J. Armstrong (eds.), American Chemical Society, Washington, DC, pp. 36-40.
86. Nagahara, A., Benjamin, H., Storkson, J., Krewson, J., Sheng, K., Liu, W., and Pariza, M. W. 1992. Inhibition of benzo[a]pyrene-induced mouse forestomach neoplasia by a principal flavor component of Japanese-style fermented soy sauce. *Cancer Res.* 52:1754-1756.
87. Pariza, M. W. 1992. Foods of new biotechnology vs traditional products: microbiological aspects. *Food Technol.* 46(3):100-102.
88. Pariza, M. W. 1992. Designer foods: effects on development of cancer. *J. Natl. Cancer Inst. Monographs* 12:105-107.
89. Pariza, M. W. 1992. Chemoprevention by conjugated dienoic isomers of linoleic acid. In: *Cancer Chemoprevention*, L. Wattenberg, M. Lipkin, C. W. Boone and G. J. Kelloff (eds.), CRC Press, Inc., Boca Raton, FL, pp. 279-283.
90. Chin, S. F., Liu, W., Storkson, J. M., Ha, Y. L. and Pariza, M. W. 1992. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *J. Food Comp. Anal.* 5:185-197.
91. Foster, E. M. and Pariza, M. W. 1993. Food safety: federal inspection programs. Commissioned by the Council on Scientific Affairs, American Medical Association. *Arch. Fam. Med.* 2:210-214.
92. Cook, M. E., Miller, C. C., Park, Y. and Pariza, M. 1993. Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. *Poultry Sci.* 72:1301-1305.
93. Pariza, M. W. 1993. Diet and cancer: where do matters stand? Commissioned by the Council on Scientific Affairs, American Medical Association, Chicago, IL. *Arch. Intern. Med.* 153:50-56.
94. Chin, S. F., Storkson, J. M., and Pariza, M. W. 1993. Conjugated dienoic derivatives of linoleic acid. Chapter 21. In: *Food Flavor and Safety: Molecular Analysis and Design*, A. M. Spanier, H. Okai, and M. Tamura (eds.), American Chemical Society, Washington, DC, pp. 262-271.
95. Pariza, M. W. 1993. Chapter 88: Diet, cancer, and food safety. In: *Modern Nutrition in Health and Disease*, 8th Edition. M. E. Shils, J. A. Olson, and M. Shike (eds.), Lea and Febiger, Philadelphia, pp. 1545-1558.

000393

96. Pariza, M. W. 1993. CLA and HEMF: newly recognized anticarcinogenic antioxidants. In: *Active Oxygens, Lipid Peroxides, and Antioxidants*, K. Yagi (ed.), Japan Sci. Soc. Press, Tokyo/CRC Press, Boca Raton, FL, pp. 359-365.
97. Pariza, M. W. 1994. Chapter 28. Fermentation-derived anticarcinogenic flavor compound. In: *Food Phytochemicals for Cancer Prevention I*, American Chemical Society Symposium Series 546, M. T. Huang, T. Osawa, C. T. Ho, and R. T. Rosen (eds.), American Chemical Society, Washington, DC, pp. 349-352.
98. Bonorden, W. R. and Pariza, M. W. 1994. Antioxidant nutrients and protection from free radicals. In: *Nutritional Toxicology, Target Organ Toxicology Series*, F. N. Kotsonis, M. Mackey, and J. J. Hjelle (eds.), Raven Press, Ltd., New York, pp. 19-48.
99. Miller, C. C., Park, Y., Pariza, M. W., and Cook, M. E. 1994. Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection. *Biochem. Biophys. Res. Commun.* 198:1107-1112.
100. Lee, K. N., Kritchevsky, D., and Pariza, M. W. 1994. Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* 108:19-25.
101. Chin, S. F., Storkson, J. M., Liu, W., Albright, K. J., and Pariza, M. W. 1994. Conjugated linoleic acid (9, 11- and 10, 12-octadecadienoic acid) is produced in conventional but not germ-free rats fed linoleic acid. *J. Nutr.* 124:694-701.
102. Chin, S. F., Storkson, J. M., Albright, K. J., Cook, M. E., and Pariza, M. W. 1994. Conjugated linoleic acid is a growth factor for rats as shown by enhanced weight gain and improved feed efficiency. *J. Nutr.* 124:2344-2349.
103. Yurawecz, M. P., Hood, J. K., Roach, J. A. G., Mossoba, M. M., Daniels, D. H., Ku, Y., Pariza, M. W. and Chin, S. F. 1994. Conversion of allylic hydroxy oleate to conjugated linoleic acid and methoxy oleate by acid-catalyzed methylation procedures. *J. Am. Oil Chem. Soc.* 71:1149-1155.
104. Conn, R. E., Kolstad, J. J., Borzelleca, J. F., Dixler, D. S., Filer Jr., L. J., LaDu, Jr., B. N., and Pariza, M. W. 1995. Safety assessment of polylactide (PLA) for use as a food-contact polymer. *Fd. Chem. Toxic.* 33(4):273-283.
105. Cohen, S. M., Clydesdale, F. M., Winter, C., Graham, J. D., Weil Jr., W. B., Kroger, M., Pariza, M. W., Crawford, L. M., Avery, D., Scheuplein, R. J. and Weisberger, E. 1995. Delaney Reform. (Letter.) *Science* 268:1829-1830.
106. Liew, C., Schut, H. A. J., Chin, S. F., Pariza, M. W. and Dashwood, R. H. 1995. Protection of conjugated linoleic acids against 2-amino-3-methylimidazo[4,5-f]quinoline-induced colon carcinogenesis in the F344 rat: a study of inhibitory mechanisms. *Carcinogenesis* 16:3037-3043.

107. Pariza, M. W. 1996. Chapter 57: Toxic substances in foods. In: *Present Knowledge in Nutrition, Seventh Edition*. E. E. Ziegler and L. J. Filer, Jr. (eds.), ILSI Press, Washington, DC, pp. 563-573.
108. Pariza, M. W. 1996. Chapter 3: Dietary lipids and cancer: Lessons from the past, directions for the future. In: *Food Lipids and Health*. IFT Basic Symposium Series. R. E. McDonald and D. B. Min (eds.), Marcel Dekker, Inc., New York, pp. 35-41.
109. Pariza, M. W. 1996. Diet in infancy and cancer development. In: *Infant Nutrition in the Prevention of Chronic Pathology*. Third International Symposium held in Alicante, Spain on September 20, 1995. M. Moya, G. Sawatzki, A. Motulsky and J. Morán (eds.), Ediciones Ergon, S.A., Madrid, Spain, pp. 15-20.
110. Pariza, M. W. 1996. Conjugated linoleic acid: A unique anticarcinogenic fatty acid in dairy products. In: *Yogurt: Myth Versus Reality*. Proceedings of a conference presented by the National Yogurt Association held in Washington, DC on October 26-27, 1993. D. Curtis (ed.), National Yogurt Association, McLean, Virginia, pp. 111-116.
111. Estabrook, R. W., Birt, D., Carlson, G. P., Cohen, S. M., Conn, E. E., Farnsworth, N. R., Gaylor, D. W., Hall, R. L., Higginson, J., Hodgson, E., Kolonel, L. N., Krewski, D., McQueen, C. A., Pariza, M. W., Sipes, I. G., Wagner, B., Watkins, P. B., Weinstein, I. B., and Zeise, L. 1996. *Carcinogens and Anticarcinogens in the Human Diet*. National Academy Press, Washington, DC.
112. Pariza, M. W. 1996. Toxic substances. In: *Food Chemistry, Third Edition*. O. R. Fennema (ed.), Marcel Dekker, Inc., New York, pp. 825-840.
113. Lee, K. N., Pariza, M. W., and Ntambi, J. M. 1996. Differential expression of hepatic stearoyl-CoA desaturase gene 1 in male and female mice. *Biochem. Biophys. Acta* 1304:85-88.
114. Pariza, M. W. 1997. Conjugated linoleic acid, a newly recognised nutrient. *Chem. & Ind.* 12:464-466 (June 16 issue).
115. Hallagan, J. B., La Du, B. N., Pariza, M. W., Putnam, J. M., and Borzelleca, J. F. 1997. Assessment of Cassia Gum. *Fd. Chem. Toxic.* (Review Section) 35:625-632.
116. Pariza, M. W. 1997. Conjugated linoleic acid: An anticarcinogenic nutrient. In: *Food Factors for Cancer Prevention*. H. Ohigashi, T. Osawa, J. Terao, S. Watanabe, and T. Yoshikawa (eds.), Springer-Verlag Tokyo, Inc., Tokyo, Japan, pp. 365-366.
117. Park, Y., Albright, K. J., Liu, W., Storkson, J. M., Cook, M. E., and Pariza, M. W. 1997. Effect of conjugated linoleic acid on body composition in mice. *Lipids* 32:853-858.

118. Kataoka, S., Liu, W., Albright, K., Storkson, J., and Pariza, M. 1997. Inhibition of benzo[a]pyrene-induced mouse forestomach neoplasia and reduction of H₂O₂ concentration in human polymorphonuclear leucocytes by flavour components of Japanese-style fermented soy sauce. *Fd. Chem. Toxic.* 35:449-457.
119. Pariza, M. W. 1997. Animal studies: summary, gaps, and future research. *Am. J. Clin. Nutr.* 66(suppl):1539S-40S.
120. Garcia, H. S., Storkson, J. M., Pariza, M. W., and Hill Jr., C. G. 1998. Enrichment of butteroil with conjugated linoleic acid via enzymatic interesterification (acidolysis) reactions. *Biotech. Letters* 20:393-395.
121. Cook, M. E. and Pariza, M. W. 1998. The role of conjugated linoleic acid (CLA) in health. *Int. Dairy J.* 8:459-462.
122. Park, Y. and Pariza, M. W. 1998. Evidence that commercial calf and horse sera can contain substantial amounts of *trans*-10,*cis*-12 conjugated linoleic acid. *Lipids* 33:817-819.
123. Lee, K. N., Pariza, M. W., and Ntambi, J. M. 1998. Conjugated linoleic acid decreases hepatic stearoyl-CoA desaturase mRNA expression. *Biochem. Biophys. Res. Commun.* 248:817-821.
124. Pariza, M. W., Ponakala, S. V., Gerlat, P.A., and Andress, S. 1998. Predicting the functionality of direct food additives. *Food Technol.* 52(11):56-60.
125. Dhiman, T. R., Helmink, E. D., McMahon, D.J., Fife, R. L., and Pariza, M. W. 1999. Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds. *J. Dairy Sci.* 82: 412-419.
126. Park, Y., Storkson, J. M., Albright, K. J., Liu, W., and Pariza, M. W. 1999. Evidence that the *trans*-10,*cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids* 34:235-241.
127. Park, Y., Albright, K. J., Storkson, J. M., Liu, W., Cook, M. E., and Pariza, M. W. 1999. Changes in body composition in mice during feeding and withdrawal of conjugated linoleic acid. *Lipids* 34:243-248.
128. Yurawecz, M. P., M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson (eds.). 1999. *Advances in Conjugated Linoleic Acid Research, Volume 1*, AOCS Press, Champaign, IL.
129. Pariza, M. W. 1999. Chapter 2: The biological activities of conjugated linoleic acid. In: *Advances in Conjugated Linoleic Acid Research, Volume 1*. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson (eds.), AOCS Press, Champaign, IL, pp. 12-20.

000396

130. Cook, M. E., DeVoney, D., Drake, B., Pariza, M. W., Whigham, L., and Yang, M. 1999. Chapter 17: Dietary control of immune-induced cachexia: conjugated linoleic acid and immunity. In: *Advances in Conjugated Linoleic Acid Research, Volume 1*. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson (eds.), AOCS Press, Champaign, IL, pp. 226-237.
131. Dhiman, T. R., Anand, G. R., Satter, L. D., and Pariza, M. W. 1999. Conjugated linoleic acid content of milk from cows fed different diets. *J. Dairy Sci.* 82:2146-2156.
132. Pariza, M. W., Park, Y., and Cook, M. E. 1999. Conjugated linoleic acid and the control of cancer and obesity. *Toxicol. Sci.* 52 (Supplement):107-110.
133. Pariza, M. W., Park, Y., and Cook, M. E. 2000. Mechanisms of action of conjugated linoleic acid: evidence and speculation. *Proc. Soc. Exp. Biol. Med.* 223:8-13.

List A-4

000398

CURRICULUM VITAE

TIMOTHY DUKES PHILLIPS, M.S., Ph.D., Fellow ATS

POSITION AND ADDRESS Professor of Toxicology

BIRTHDATE AND PLACE:

CURRENT EMPLOYMENT:

<u>Date</u>	<u>Position(s) (Rank)</u>	<u>Department</u>
1998-present	Professor	Faculty of Toxicology (Texas A&M University)
1996-1998	Professor & Chair	Faculty of Toxicology (Texas A&M University)
1990-present	Professor	Faculty of Toxicology (Texas A&M University)
1990-present	Professor	Faculty of Food Science (Texas A&M University)
1990-present	Professor	Anatomy & Public Health (Texas A&M University)

PREVIOUS ACADEMIC EMPLOYMENT:

<u>Date</u>	<u>Position</u>	<u>Institution</u>
1986-1990	Professor	Veterinary Public Health Food Science and Technology (TAMU)
1982-1990	Adjunct Professor	Pharmacology and Toxicology University of TX Medical Center, Galveston
1982-1986	Associate Professor	Veterinary Public Health (TAMU) Food Science and Technology (TAMU)

000399

PREVIOUS ACADEMIC EMPLOYMENT (cont.):

<u>Date</u>	<u>Position</u>	<u>Institution</u>
1979-1981	Assistant Professor	Veterinary Public Health Food Science and Technology Texas A&M University
1977-1979	Research Associate	Medical Pharmacology and Toxicology University of Mississippi Medical Center
1975-1976	Robert A. Welch Foundation Fellow	Institute for Rehabilitation Research Baylor College of Medicine, Houston
1972-1975	National Defense & Education Act (N.D.E.A.) Fellow	Department of Chemistry University of Southern Mississippi
1971-1972	N.D.E.A. Fellow	Department of Sci. Ed. (Chemistry) University of Southern Mississippi

EDUCATIONAL BACKGROUND:

<u>Degrees</u>	<u>Major</u>	<u>Received From</u>	<u>Date</u>
Ph.D.	Chemistry	Univ. of Southern Mississippi	1975
M.S.	Sci. Ed./Chemistry	Univ. of Southern Mississippi	1972
B.S.	Gen. Science	Mississippi State University	1970

BOARD CERTIFICATION:

Fellow, Academy of Toxicological Sciences (January 10, 2000)

PROFESSIONAL INTERESTS:

Food Safety and Environmental Toxicology - Development and evaluation of chemical and biological methods to detect and detoxify food-borne and environmental toxins and microbes; molecular and kinetic assessment of the mechanisms involved in the toxic actions/interactions of mycotoxins, corresponding metabolites and polysubstituted derivatives; multifunctional detoxifying clays and matrix-immobilized clay composites for the sorption and inactivation of hazardous mycotoxins and environmental contaminants; molecular modeling and thermodynamics of surface-toxin interactions.

PROFESSIONAL ACTIVITIES AND AWARDS:

Invited Speaker: Philippine Society of Nutrition, Manila, the Philippines, 1999
 Invited Speaker: Vicam International Meeting, Boston, MA, 1999
 Invited Speaker: U.S. Feed Grain Association; Dominican Republic, 1999

Gordon Conference on Mycotoxins and Phycotoxins, 1999
Invited Speaker: FAO/WHO/UNEP Congress on Mycotoxins, Tunis, Tunisia, 1999
Pfizer Award for Excellence in Research, 1998
USDA NRICGP Study Panel (Animal Health), 1998
Chair, Intercollegiate Faculty of Toxicology, Texas A&M University, 1996-1998
Invited Speaker: FDA, Washington, D.C., 1998
Invited Speaker: NIEHS Superfund Basic Research Program, New York, NY, 1998
Invited Speaker: SOT Contemporary Concepts in Toxicology, Reston, VA, 1998
Board of Scientific Advisors, American Council on Science and Health, 1986-present
Institute of Food Science and Engineering Symposium: Food Safety Moderator, 1997
Council for Agricultural Science and Technology: Task Force on Mycotoxins, 1997-98
Society of Toxicology, CE course: Food Safety Specialty Section, Cincinnati, Ohio, 1997
Invention Disclosure: *Hydraulically Conductive Product Containing Clay*, 1998
Invention Disclosure: *Equilibrium Adsorption of zearalenone by organophilic clay*, 1997
Invited Speaker: Taipei, Taiwan; Bangkok, Thailand; and Manila, the Philippines, 1996
Invited Speaker: Associated Milk Producers, Inc., Dallas, Texas, 1996
Invited Speaker: Association of Milk, Food and Environ. Sanitarians, Austin, TX, 1996
Invited Speaker: UT Health Science Center, Toxicol. and Pharmacol., Houston, TX, 1996
Invited Speaker: UM Medical Center, Toxicology and Pharmacology, Jackson, MS, 1996
Invited Speaker: Food Science and Engineering, Technology transfer Symposium, 1996
Invention Disclosure: Clay-based dehalogenation of SuperFund chemicals (TAMU), 1995
Invention Disclosure: ClayPac technology, Technology Licensing Office (TAMU), 1995
Director, Minority Recruitment (NIH Toxicology Training Grant), 1995-present
Technology License Agreement: VICAM, Watertown, Massachusetts, 1995-1996
Invited Speaker: Society of Toxicology (Gulf Coast Chapter), San Antonio, Texas, 1995
Invited Speaker: Aflatoxin Conference (TAES & TAEX), Corpus Christi, Texas, 1995
Invited Speaker: International Mycotoxin Symposium (FACTA), Curitiba, Brazil, 1995
Invited Speaker: American Feed Industry Insurance Co., St. Louis, Missouri, 1995
Invited Speaker: American Society of Agronomy, St. Louis, Missouri, 1995
Invited Speaker: Roche, Mexico City, Guadalajara and Monterrey, Mexico, 1995
Invited Speaker: World Health Organization/FAO/European Union, Lisbon, Portugal, 1994
Invited Speaker: National Veterinary Research Institute, Pulawy, Poland, 1994
Invited Speaker: NutriBasics (DuPont), Thailand, Taiwan, Phillipines, Maylasia, 1993
Invited Speaker: Hoffmann-La Roche, Brazil, Costa Rica, Mexico and Peru, 1993
SmithKline Beecham Award for Research, 1993
Scientific Advisory Panel, Ralston Purina International, St. Louis, Missouri, 1992
Invited Speaker: Pennington Symposium on Aflatoxin, Baton Rouge, Louisiana, 1990
Invited Speaker: Congressional Symposium on Aflatoxin, Washington, D.C., 1990
Committee on Agriculture, U.S. House of Representatives, (CAST report), 1990
Director of Minority Recruitment, Toxicology Program, TAMU, 1989-Present
Engelhard Achievement Award for the Discovery of NovasilTM, 1989
TAMU Faculty Distinguished Achievement Award in Research, 1988
Council for Agricultural Science and Technology (CAST) Task Force, 1988
Elected office: President, Gulf Coast Chapter of SOT, 1986-1987
Texas A&M University System Award in Research, 1986
Faculty Achievement Award in Research (TVMA), 1985
Editorial Board, "Environmental Toxin Reviews", 1984-1996
Chairman, USDA S-175 Technical Committee on Mycotoxins, 1984-85
Advisory Panel, National Space Technology Laboratories, 1984-86
Phi Zeta Veterinary Honor Society, 1984
Analytical Separations Advisory Council (Water's Associates, Inc.), 1984

International Program Activities (USAID), Senegal, West Africa, 1983-1996
Secretary/Treasurer, USDA S-132 Technical Committee on Mycotoxins, 1982-83
Nominating Committee, Society of Toxicology, 1982-83
Robert A. Welch Foundation Postdoctoral Fellowship, 1975-76
National Defense and Education Act Predoctoral Fellowship, 1971-75
Member, Society of Toxicology (SOT); Section on Food Safety (Awards Committee)
Member, FASEB, American Society of Pharmacology and Experimental Therapeutics
Member, American Chemical Society; Speciality Section on Chemical Toxicology

ACADEMIC COMMITTEE EXPERIENCE:

System Intellectual Property Committee, Texas A&M University, 1991-1998
Executive Committee, Faculty of Toxicology, Texas A&M University, 1990-present
Executive Committee, Faculty of Food Science, Texas A&M University
Center for Food Safety Steering Committee, 1994-present
Molecular Science and Engineering Steering Committee, TAMU, 1994-1996
Search Committee (Food Safety/Toxicology), VTAN/VTPH, 1990 (Chair)
Graduate Instruction Committee, College of Veterinary Medicine, 1990 (Chair)
Search Committee (Associate Dean for Research, CVM), 1989-1990 (Chair)
Faculty Distinguished Achievement Awards Selection Committee, TAMU, 1990
Committee on Workshops and Special Sessions, TAES Staff Conference, 1990
Graduate Programs Committee, Toxicology Program, Texas A&M University
Membership Committee, Toxicology Program, Texas A&M University
Industrial Liaison Committee, Toxicology Program, Texas A&M University
College of Veterinary Medicine, PEW Advisory Committee, CVM
Ad Hoc Committee on Research Strategies, PEW Advisory Committee, CVM
Interdepartmental Toxicology Steering Committee, TAMU
Texas A&M University Council of Principal Investigators, TAMU
Graduate Assistantship Committee, Veterinary Public Health
Graduate Student Recruiting Committee, Veterinary Public Health
Subcommittee on Graduate Education, College of Veterinary Medicine
Research Working Groups (TAES Staff Conference Committee)
Secretary, Graduate Instruction Committee, College of Veterinary Medicine
Relocation Committee, Veterinary Public Health
TAES Staff Conference Committee (Research working groups)
Graduate Studies Committee, Veterinary Public Health
Research Advisory Council, College of Veterinary Medicine
Graduate Instruction Committee, College of Veterinary Medicine
Safety, Health and Security Committee, Veterinary Public Health
ERA Study Section

CURRENT RESEARCH (GRANTS/CONTRACTS):

DHHS/PHS/NIEHS-15617-6918-6215 (Safe). *Superfund Basic Research Program (Detection and Ranking of Developmental Hazards Associated with Complex Mixtures of Chemical Wastes)*; \$302,388; (04/96-03/00); CoPI; Location: Texas A&M University; Personnel Support = 2 graduate students and 1 postdoctoral research associate.

USDA NRI CSRS-9703230 (Phillips). *Clay-based strategies for the prevention of mycotoxicoses in animals*; \$165,000; (09/97-08/00); PI; Location: Texas A&M University; Personnel Support = 2 graduate students.

Engelhard Chemical Corporation- TAES 402905-98 (Phillips) *Development of clay-based technologies for the detoxification of mycotoxins*; \$ [REDACTED]; (09/98-08/01); PI; Location: Texas A&M University; Personnel Support = 1 student worker.

Texas Department of Health, Division of Milk and Dairy-IAC 1459-32790 (Phillips). *Detection of Aflatoxins in Milk and Feed Samples*; (recurrent variable funds); PI; Location: Texas A&M University; Personnel Support = 1 student worker.

DHHS/PHS/NIEHS 1T32-ES07273-Training Grant (Safe). *Toxicology of environmental contaminants*; \$130,726; (07/97-06/02); CoI; Location: Texas A&M University; Personnel Support = 1 graduate student.

Engelhard/DuCoa, Inc. (Phillips). *License Agreement with TAES; NovaSil™; Method for Inactivating mycotoxins in the gastrointestinal tract of animals*; Royalty Payments to Research; (01/89-1/2001); PI; Location: Texas A&M University and Cleveland, Ohio.

USDA (Small Business Innovation Research, Phase II). *Destruction of aflatoxins in grain using gaseous ozone*; \$255,000; CoPI; Location: Lynntech, Inc. and Texas A&M University.

Texas Agricultural Experiment Station (TAES) Hatch Project TEXO6215 (Phillips). *Clay-based strategies for the prevention of mycotoxicoses*; PI; Location: Texas A&M University; Personnel Support = 1 graduate student and salary support for TDP.

DHHS/PHS/NIH 1 P30-ES09106 (Safe). *Center for Environmental and Rural Health*; \$500,000/year; (5/98-04/01); Investigator in the Cellular and Molecular biology core of this project; Location: Texas A&M University.

PENDING (2000) RESEARCH GRANTS/CONTRACTS:

National Science Foundation (Phillips). *Novel clay-based composites for sorption and prevention of toxic chemical byproducts of chlorination and exogenous toxins and microbes during processing of drinking water*; \$665,478; (01/00 - 12/03); PI; Location: Texas A&M University.

DHHS/PHS/NIEHS (Safe). Superfund Basic Research Program (Chemical Intervention Strategies); \$427,356 (04/00 - 03/05); CoPI; Location: Texas A&M University.

Engelhard Chemical Corporation (Phillips). *Clay-based technologies for the control of microbial hazards in food and water*; Technology License and research funds; PI; Location: Texas A&M University.

World Bank (Williams/Phillips). *Use of [REDACTED] adsorbents to provide protection from aflatoxins in African populations*; \$ [REDACTED]; (04/00 - 03/01); CoPI; Location: Ghana, West Africa.

REFEREED SCIENTIFIC PUBLICATIONS:

1. Toom, P. M. and T. D. Phillips: 1974. Effects of purified components of jellyfish toxin (*Stomolophus meleagris*) on active sodium transport. *Toxicon*. 13:261-271.
2. Toom, P.M., T.D. Phillips, and R. B. Koch: 1976. Effects of purified components of jellyfish toxin (*Stomolophus meleagris*) on adenosinetriphosphatase activities. *Biochem. Pharmacol.* 25:551-555.
3. Phillips, T.D. and A.W. Hayes: 1977. Effects of patulin on adenosine-triphosphatase activities in the mouse. *Toxicol. Appl. Pharmacol.* 42:175-187.
4. Desaiyah, D., T.D. Phillips, A.W. Hayes, and I.K. Ho: 1978. Effects of the aflatoxins on ATPase activities in mouse and rat liver. *J. Environ. Sci. Hlth.* 12:277-283.
5. Hayes, A.W., R.E. King, P.D. Unger, T.D. Phillips, J. Hatkin, and J.N. Bowen: 1978. Aflatoxicosis in swine. *Amer. J. Vet. Med.* 172:1295-1297.
6. Phillips, T.D. and A.W. Hayes: 1978. Effects of patulin on the kinetics of substrate and cationic ligand activation of adenosine triphosphatase in mouse brain. *J. Pharmacol. Exp. Ther.* 205:606-616.
7. Phillips, T.D., A.W. Hayes, I.K. Ho, and D. Desaiyah: 1978. Effects of rubratoxin B on the kinetics of cationic and substrate activation of (Na⁺-K⁺)-ATPase and p-nitrophenyl phosphatase. *J. Biol. Chem.* 253:3487-3493.
8. Siraj, M. Y., T.D. Phillips, and A. W. Hayes: 1978. Interaction of mycotoxins with copper-folin reagent. *J. Food Protect.* 41:370-372.
9. Toom, P.M. and T.D. Phillips: 1978. Effects of purified components of jellyfish toxin on adenosine triphosphatase activities. In *Toxins, Animal, Plant and Microbial*, P. Rosenberg (ed.), Pergamon Press, pp. 527- 538.
10. Unger, P.D., T.D. Phillips, and A.W. Hayes: 1978. Conversion of rubratoxin B to its carboxylic acid derivative and its effect on ATPase activity and toxicity to mice. *Fd. Cosmet. Toxicol.* 16:463-467.
11. Chan, P.K., T.D. Phillips, and A.W. Hayes: 1979. Effects of penicillic acid on brain, liver, and kidney ATPase activities in the mouse. *Toxicol. Appl. Pharmacol.* 49:365-372.
12. Hayes, A.W., T.D. Phillips, W.L. Williams, and A. Ciegler: 1979. Acute toxicity to patulin. *Toxicology* 13:91-100.
13. Phillips, T.D. and A.W. Hayes: 1979. Inhibition of electrogenic sodium transport across toad urinary bladder by the mycotoxin patulin. *Toxicology* 13:17-24.
14. Phillips, T.D. and A.W. Hayes: 1979. Structural modification of polyfunctional rubratoxin B: Effects on mammalian adenosine triphosphatase. *J. Environ. Path. and Toxicol.* 2(3):853-860.

15. Hanna, G.D., T.D. Phillips, L.F. Kubena, S.J. Cysewski, G.W. Ivie, N.D. Heidelbaugh, D.W. Witzel, and A.W. Hayes: 1980. High pressure liquid chromatographic determination of penicillic acid in chicken tissues. *Poult. Sci.* 60(10):2246-2252.
16. Kubena, L.F., T.D. Phillips, D.A. Witzel and N.D. Heidelbaugh: 1980. Influence of various levels of vanadium on female laying strain chickens. *Poult. Sci.* 59(7):1628-1629.
17. Phillips, T.D., P.K. Chan, and A.W. Hayes: 1980. Inhibitory characteristics of the mycotoxin penicillic acid on (Na⁺-K⁺) adenosine triphosphatase and p-nitrophenyl phosphatase activity. *Biochem. Pharmacol.* 29:19-26.
18. Phillips, T.D., M.Y. Siraj and A.W. Hayes: 1980. Effects of mycotoxins on mixed function oxidase and adenosine triphosphatase systems in neonatal rats. I. Aflatoxin B1/rubratoxin B. *Fd. Cosmet. Toxicol.* 18:261-266.
19. Kubena, L.F., T.D. Phillips, D.A. Witzel and N.D. Heidelbaugh: 1981. Influence of penicillic acid and ochratoxin A on various parameters in the growing chick. *Poult. Sci.* 60(7):1680-1681.
20. Phillips, T.D., G.W. Ivie, N.D. Heidelbaugh, L.F. Kubena, S.J. Cysewski, A.W. Hayes, and D.A. Witzel: 1981. Confirmation of penicillic acid by high pressure liquid and gas-liquid chromatography. *J. Assoc. Off. Anal. Chem.* 64(1):162-165.
21. Shepherd, E.C., T.D. Phillips, G.N. Joiner, L.F. Kubena, and N.D. Heidelbaugh: 1981. Ochratoxin A and penicillic acid interaction in mice. *J. Environ. Sci. Health.* B16(5):557-573.
22. Siraj, M.Y., T.D. Phillips, and A.W. Hayes: 1981. Effects of mycotoxins on mixed function oxidase and adenosine triphosphatase systems in neonatal rats. II. Ochratoxin/citrinin. *J. Environ. Sci. Health.* 8(1-2):131-141.
23. Parker, R.W., T.D. Phillips, L.F. Kubena, L.H. Russell and N.D. Heidelbaugh: 1982. Inhibition of pancreatic carboxypeptidase A: A possible mechanism of interaction between penicillic acid and ochratoxin A. *J. Environ. Sci. Health.* B17(2):77-91.
24. Phillips, T.D., B.R. Nechay, L.F. Kubena, N.D. Heidelbaugh, E.C. Shepherd, A.F. Stein, S.L. Neldon and A.W. Hayes: 1982. Vanadium induced inhibition of renal (Na⁺-K⁺)-adenosine triphosphatase in the chicken after chronic dietary exposure. *J. Toxicol. Environ. Health.* 9(4):651-661.
25. Shepherd, E.C., T.D. Phillips, A.W. Hayes and N.D. Heidelbaugh: 1982. High pressure liquid chromatographic determination of aflatoxins using radial compression separation. *J. Assoc. Off. Anal. Chem.* 65(3):665-671.
26. Turner, G.V., T.D. Phillips, N.D. Heidelbaugh and L.H. Russell: 1982. A high pressure liquid chromatographic method for the analysis of zearalenone in chicken blood. *J. Environ. Sci. Health.* B17(4):297-309.
27. Kubena, L.F., R.B. Harvey, S.L. Lovering and T.D. Phillips: 1983. A chick model for impaired renal function. *Poult. Sci.* 62(1):47-50.

28. Kubena, L.F. and T.D. Phillips: 1983. Toxicity of vanadium in female leghorn chickens. *Poult. Sci.* 61(1):47-50.
29. Kubena, L.F., T.D. Phillips, C.R. Creger, D.A. Witzel and N.D. Heidelbaugh: 1983. Toxicity of ochratoxin A and tannic acid to growing chicks. *Poult. Sci.* 62:1786-1792.
30. Phillips, T.D., B.R. Nechay and N.D. Heidelbaugh: 1983. Vanadium: chemistry and the kidney. *Fed. Proc.* 42(13):2969-2973.
31. Phillips, T.D., A.F. Stein, G.W. Ivie, L.F. Kubena and N.D. Heidelbaugh: 1983. High pressure liquid chromatographic determination of an O-methyl, methylester derivative of ochratoxin A. *J. Assoc. Off. Anal. Chem.* 66(3):570-576.
32. Turner, G.V., T.D. Phillips, N.D. Heidelbaugh and L.H. Russell: 1983. High pressure liquid chromatographic determination of zearalenone in chicken tissues. *J. Assoc. Off. Anal. Chem.* 66(1):102-104.
33. Geerling, S., H.H. Mollenhauer, A.F. Stein and T.D. Phillips: 1984. Effect of partial nephrectomy and ochratoxin A on renal structure: A light and electron microscope study. *T.S.E.M.J.* 15:20-24.
34. Harvey, R.B., L.F. Kubena, S.L. Lovering, and T.D. Phillips: 1984. A chick model for impaired renal function. *Poult. Sci.* 63:1920-1924.
35. Kubena, L.F., T.D. Phillips, D.A. Witzel, and N.D. Heidelbaugh: 1984. Toxicity of ochratoxin A and penicillic acid to chicks. *Bull. Environ. Contam. Toxicol.* 32(6):711-716.
36. Mayura, K., W.O. Berndt, and T.D. Phillips: 1984. Effect of simultaneous prenatal administration of ochratoxin A and citrinin on prenatal mortality in the rat. *J. Toxicol. Environ. Health.* 13:553-561.
37. Mayura, K., R. Parker, W.O. Berndt, and T.D. Phillips: 1984. Ochratoxin A-induced teratogenesis the rats: Partial protection by phenylalanine. *Appl. and Env. Micro.* 48:1186-1188.
38. Mayura, K., A.F. Stein, W.O. Berndt, N.D. Heidelbaugh and T.D. Phillips: 1984. Teratogenic effects of ochratoxin A in rats with impaired renal function. *Toxicology* 32:277-285.
39. Shepherd, E.C., T.D. Phillips, T.R. Irvin, S.H. Safe, L. Robertson: 1984. Aflatoxin B₁ metabolism in the rat: polyhalogenated biphenyl enhanced conversion to aflatoxin M₁. *Xenobiotica* 14:741-750.
40. Stein, A.F., S. Geerling, H.H. Mollenhauer, N.D. Heidelbaugh and T.D. Phillips: 1984. Effects of ochratoxin A in the partially nephrectomized rat. *J. Toxicol. Environ. Health.* 14(4):535-550.
41. Halvorson, M.R., T.D. Phillips, S.H. Safe, and L.W. Robertson: 1985. Metabolism of aflatoxin B₁ by rat hepatic microsomes induced by polyhalogenated biphenyl congeners. *Appl. Environ. Microbiol.* 49(4):882-886.

42. Harvey, R.B., L.F. Kubena, S.L. Lovering and T.D. Phillips: 1985. Ketamine/xylazine anesthesia for chickens. *Avian/Exotic Practice*. 2:6-7.
43. Kubena, L.F., R.B. Harvey, O.J. Fletcher, T.D. Phillips, H.H. Mollenhauer, D.A. Witzel, and N.D. Heidelbaugh: 1985. Toxicity of ochratoxin A and vanadium to growing chicks. *Poult. Sci.* 64(4):620-628.
44. Kubena, L. F., S. P. Swanson, R. B. Harvey, O. J. Fletcher, L. D. Rowe and T. D. Phillips: 1985. Effects of feeding deoxynivalenol (DON, vomitoxin) contaminated wheat to growing chicks. *Poult. Sci.* 64(9):1649-1655.
45. Stein, A.F., T.D. Phillips, L.F. Kubena and R.B. Harvey: 1985. Renal secretion and reabsorption as factors in ochratoxicosis: Effects of probenecid on nephrotoxicity. *J. Toxicol. Environ. Health* 16:593-605.
46. Ballinger, M.B., T.D. Phillips and L.F. Kubena: 1986. Assessment of the distribution and elimination of ochratoxin A in the pregnant rat. *J. of Food Safety*. 8:11-24.
47. Harvey, R.B., L.F. Kubena, D.E. Corrier, T.D. Phillips, and N.D. Heidelbaugh: 1986. Effects of deoxynivalenol (DON, vomitoxin) in a wheat ration fed to growing lambs. *Am. J. Vet. Res.* 47(7):1630-1632.
48. Harvey, R.B., L.F. Kubena, S.L. Lovering, H.H. Mollenhauer and T.D. Phillips: 1986. Acute toxicity of uranyl nitrate to growing chicks: A pathophysiologic study. *Bull. Environ. Contam. Toxicol.* 37:907-915.
49. Harvey, R.B., L.F. Kubena, T.D. Phillips, and N.D. Heidelbaugh: 1986. Validation of impaired renal function chick model with uranyl nitrate. *Bull. Environ. Cont. Toxicol.* 36(1):67-72.
50. Huff, W.D., L.F. Kubena, R.B. Harvey, W.M. Hagler, Jr., S.P. Swanson, T.D. Phillips, and C.R. Creger: 1986. Individual and combined effects of aflatoxin and deoxynivalenol (DON, vomitoxin) in broiler chickens. *Poult. Sci.* 65:1291-1298.
51. Kubena, L.F., R.B. Harvey, T.D. Phillips and O.J. Fletcher: 1986. Influence of ochratoxin A and vanadium on various parameters in growing chicks. *Poult. Sci.* 65:1671-1678.
52. Sylvia, V.L., T.D. Phillips, B.A. Clement, J.L. Green, L.F. Kubena and N.D. Heidelbaugh: 1986. Determination of deoxynivalenol (vomitoxin) by high pressure liquid chromatography with electrochemical detection. *J. of Chromatography* 362(1):79-85.
53. Haake, J.M., S. Safe, K. Mayura and T.D. Phillips: 1987. Aroclor 1254 as an antagonist of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicology Letters* 38:299-306.
54. Harvey, R.B., L.F. Kubena, D.B. Lawhorn, O.J. Fletcher and T.D. Phillips: 1987. Feed refusal in swine fed ochratoxin-contaminated grain sorghum: Evaluation of toxicity in chicks. *J. Am. Vet. Med. Assoc.* 190:673-675.

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55. Kubena, L.F., R.B. Harvey, D.E. Corrier, W.E. Huff and T.D. Phillips: 1987. Effects of feeding deoxynivalenol (DON, vomitoxin) contaminated wheat to female white Leghorn chickens from day old through egg production. *Poult. Sci.* 66:1612-1618.
56. Kubena, L.F., R.B. Harvey, T.D. Phillips, G.M. Holman and C.R. Creger: 1987. Effects of feeding Leghorn hens diets that contain DON. *Poult. Sci.* 66:55-58.
57. Mayura, K., E.E. Smith, B.A. Clement, R.B. Harvey, L.F. Kubena and T.D. Phillips: 1987. Developmental toxicity of diacetoxyscirpenol in the mouse. *Toxicology* 45:245-255.
58. Munger, C.E., G.W. Ivie, R.J. Christopher, B.D. Hammock and T.D. Phillips: 1987. Acetylation/deacetylation reactions of T-2, acetyl T-2, HT-2, and acetyl HT-2 toxins in bovine rumen fluid in vitro. *J. Agricul. Fd. Chem.* 35(3):354-358.
59. Halvorson, M.R., Safe, S.H., Parkinson, A. and T.D. Phillips: 1988. Aflatoxin B1 hydroxylation by the pregnenolone-16 alpha-carbonitrile (PCN)-inducible form of rat liver microsomal cytochrome P-450. *Carcinogenesis* 9(11):2103-2108.
60. Harvey, R.B., D.E. Clark, W.E. Huff, L.F. Kubena, D.E. Corrier and T.D. Phillips: 1988. Suppression of serum iron-binding capacity and bone marrow cellularity in pigs fed aflatoxin. *Bull. Environ. Contam. Toxicol.* 40:576-583.
61. Harvey, R.B., W.E. Huff, L.F. Kubena, D.E. Corrier and T.D. Phillips: 1988. Progression of aflatoxicosis in growing pigs. *Am. J. Vet. Res.* 49(4):482-487.
62. Harvey, R.B., L.F. Kubena, S.A. Naqi, J.E. Gyimah, D.E. Corrier, B. Panigrahy and T.D. Phillips: 1988. Immunologic effects of low levels of Ochratoxin A in ovo: Utilization of a chick embryo model. *Avian Diseases* 31:787-791.
63. Kubena, L.F., W.E. Huff, R.B. Harvey, D.E. Corrier, T.D. Phillips and C.R. Creger: 1988. Influence of ochratoxin A (OA) and deoxynivalenol (DON) on growing chicks. *Poult. Sci.* 67:253-260.
64. Phillips, T.D., L.F. Kubena, R.B. Harvey, D.R. Taylor and N.D. Heidelbaugh: 1988. Hydrated sodium calcium aluminosilicate: High affinity sorbent for aflatoxin. *Poult. Sci.* 67:243-247.
65. Harvey, R.B., W.E. Huff, L.F. Kubena, and T.D. Phillips: 1989. Evaluation of diets co-contaminated with aflatoxin and ochratoxin fed to growing pigs. *Am. J. Vet. Res.* 50:1400-1405.
66. Harvey, R.B., L.F. Kubena, W.E. Huff, D.E. Corrier D.E. Clark and T.D. Phillips: 1989. Effects of aflatoxin, deoxynivalenol, and their combinations in the diets of growing pigs. *Am J Vet Res*, 50(4):602-607.
67. Harvey, R.B., L.F. Kubena, T.D. Phillips, W.E. Huff and D.E. Corrier: 1989. Prevention of aflatoxicosis by addition of hydrated sodium calcium aluminosilicate to the diets of growing barrows. *Am. J. Vet. Res.* 50(3):416-420.

68. Kubena, L.F., R.B. Harvey, W.E. Huff, D.E. Corrier, T.D. Phillips and G.E. Rottinghaus: 1989. Influence of ochratoxin A (OA) and T-2 toxin singly and in combination on broiler chicks. *Poult. Sci.* 68:867-872.
69. Kubena, L.F., W.E. Huff, R.B. Harvey, T.D. Phillips, and G.E. Rottinghaus: 1989. The individual and combined toxicity of deoxynivalenol and T-2 toxin in broiler chicks. *Poult. Sci.* 68:622-626.
70. Phillips, T.D. and A.W. Hayes: 1989. Techniques in membrane toxicology. In *Principles and Methods in Toxicology* (2nd Edition). A.W. Hayes (Ed.), Raven Press.
71. Harvey, R.B., L.F. Kubena, W.E. Huff, D.E. Corrier, G.E. Rottinghaus, and T.D. Phillips: 1990. Effects of treatment of growing swine with aflatoxin and T-2 toxin. *Am. J. Vet. Res.* 51:1688-1693.
72. Kubena, L., R. Harvey, W. Huff, D. Corrier, T. Phillips and G. Rottinghaus: 1990. Ameliorating properties of a hydrated sodium calcium aluminosilicate on the toxicity of aflatoxin and T-2 toxin. *Poult. Sci.* 69:1078-1086.
73. Kubena, L.F., R.B. Harvey, T.D. Phillips, D.E. Corrier and W.E. Huff: 1990. Diminution of aflatoxicosis in growing chickens by the dietary addition of a hydrated, sodium calcium aluminosilicate. *Poult. Sci.* 69:727-735.
74. Mayura, K., E.E. Smith, B.A. Clement and T.D. Phillips: 1990. Evaluation of the developmental toxicity of chlorinated phenols utilizing *Hydra attenuata* and postimplantation rat embryos in culture. *Toxicol. Appl. Pharmacol.* 108:253-266.
75. Phillips, T.D., B.A. Sarr, B.A. Clement, L.F. Kubena and R.B. Harvey: 1990. Prevention of aflatoxicosis in farm animals via selective chemisorption of aflatoxin. In *Mycotoxins, Cancer and Health* (Pennington Center Nutrition Series, Vol. 1), pp. 223-228, Louisiana State University Press, Baton Rouge and London.
76. Phillips, T.D., B.A. Clement, L.F. Kubena and R.B. Harvey: 1991. Prevention of aflatoxicosis and aflatoxin residues with HSCAS. *Vet. Human Toxicol.* 32:15-19.
77. Harvey, R.B., L.F. Kubena, T.D. Phillips, D.E. Corrier, M.H. Elissalde and W.E. Huff: 1991. Diminution of aflatoxin toxicity to growing lambs by dietary supplementation with hydrated sodium calcium aluminosilicate. *Am. J. Vet. Res.* 52:152-156.
78. Harvey, R.B., T.D. Phillips, J.A. Ellis, L.F. Kubena, W.E. Huff and D.V. Peterson: 1991. Effects of aflatoxin M₁ residues in milk by addition of hydrated sodium calcium aluminosilicate to aflatoxin-contaminated diets of dairy cows. *Am. J. Vet. Res.* 52:1556-1559.
79. Harvey, R.B., L.F. Kubena, W.E. Huff, M.H. Elissalde and T.D. Phillips: 1991. Hematologic and immunologic toxicity of deoxynivalenol (DON)-contaminated diets to growing chickens. *Bull. Environ. Contam. Toxicol.* 46:410-416.
80. Kubena, L.F., W. Huff, R.B. Harvey, A. Yersin, M. Elissalde, D. Witzel, L. Giroir, T.D. Phillips and H. Peterson: 1991. Effects of hydrated sodium calcium aluminosilicate on growing turkey poults during aflatoxicosis. *Poult. Sci.* 70:1823-1830.

81. Huff, W.E., L.F. Kubena, R.B. Harvey and T.D. Phillips: 1991. Efficacy of hydrated sodium calcium aluminosilicate to reduce the combined toxicity of aflatoxin and ochratoxin A. *Poult. Sci.*
82. Harvey, R.B., L.F. Kubena, W.E. Huff, M.H. Elissalde, and T.D. Phillips: 1991. Hematologic and immunologic toxicity of deoxynivalenol (DON) contaminated diets to growing chickens. *Bull. Environ. Contam. Toxicol.* 46:410-416.
83. Mayura, K., E.E. Smith, B.A. Clement, and T.D. Phillips: 1991. Evaluation of the developmental toxicity of chlorinated phenols utilizing *Hydra attenuata* and postimplantation rat embryos in culture. *Toxicol. Appl. Pharmacol.* 108:253-266.
84. Narasimhan, T.R., K. Mayura, B.A. Clement, S.H. Safe and T.D. Phillips: 1992. Effects of chlorinated phenols on rat embryonic and hepatic mitochondrial oxidative phosphorylation. *Environ. Toxicol. Chem.* 11:805-814.
85. Burghardt, R.C., R. Barhoumi, E.H. Lewis, R.H. Bailey, K.A. Pyle, B.A. Clement and T.D. Phillips: 1992. Patulin-induced cellular toxicity: A vital fluorescence study. *Toxicol. Appl. Pharmacol.* 112:235-244.
86. Mayura, K., C.B. Spainhour, L. Howie, S. Safe and T.D. Phillips: 1993. Teratogenicity and immunotoxicity of 3,3',4,4',5-pentachlorobiphenyl in C57BL/6 mice. *Toxicology* 77:123-131.
87. Kubena, L.F., R.B. Harvey, W.E. Huff, M.H. Elissalde, A.G. Yersin, T.D. Phillips and G.E. Rottinghaus: 1993. Efficacy of HSCAS to reduce the toxicity of aflatoxin and diacetoxyscirpenol. *Poult. Sci.* 72:51-59.
88. Harvey, R.B., L.F. Kubena, M.H. Elissalde and T.D. Phillips. 1993. Efficacy of zeolitic ore compounds on the toxicity of aflatoxin to growing broiler chickens. *Avian Diseases* 37:67-73.
89. Kubena, L.F., R.B. Harvey, T.D. Phillips and B.A. Clement. 1993. Effect of hydrated sodium calcium aluminosilicates on aflatoxicosis in broiler chicks. *Poult. Sci.* 72:651-657.
90. Yang, Y.G., K. Mayura, C.B. Spainhour, Jr., J.F. Edwards and T.D. Phillips. 1993. Evaluation of the developmental toxicity of citrinin using *Hydra attenuata* and postimplantation rat whole embryo culture. *Toxicology* 85:179-198.
91. Cathey, C.G., Z. Huang, A. Sarr, B. Clement and T.D. Phillips. 1994. Development of a minicolumn assay for the detection of aflatoxin M₁. *J. Dairy Sci.* 77:1223-1231.
92. Burghardt, R.C., R. Barhoumi, D.J. Doolittle, and T.D. Phillips. 1994. Application of cellular fluorescence imaging for in vitro toxicology testing. In: Principles and Methods of Toxicology, (A.W. Hayes, ed.) pp. 1231-1258, Raven Press, New York.
93. Phillips, T.D., B.A. Clement, and D.L. Park. 1994. Approaches to reduction of aflatoxins in foods and feeds. In: The Toxicology of Aflatoxins: Human Health, Veterinary, and Agricultural Significance (D. Eaton and J. Groopman, eds), pp. 383-406, A. Press, NY.

94. Kubena, L.F., E.E. Smith, A. Gentles, R.B. Harvey, T.S. Edrington, T.D. Phillips, and G.E. Rottinghaus. 1994. Individual and combined toxicity of T-2 toxin and cyclopiazonic acid in broiler chicks. *Poult. Sci.* 73:1390-1397.
95. Harvey, R., L. Kubena, M. Elissalde, D. Corrier, and T.D. Phillips. 1994. Comparison of two hydrated sodium calcium aluminosilicate compounds to experimentally protect growing barrows from aflatoxicosis. *J. Vet. Diagn Invest* 6:88-92.
96. Smith, E.E., T.D. Phillips, J.A. Ellis, R.B. Harvey, L.F. Kubena, J. Thompson, and G. Newton. 1994. Dietary hydrated sodium calcium aluminosilicate reduction of aflatoxin M1 residue in dairy goat milk and effects on milk production and components. *J. Anim. Sci.* 72:677-682.
97. Keller, N.P., R.A.E. Butchko, A.B. Sarr, and T.D. Phillips. 1994. A visual pattern of mycotoxin production in maize kernels by *Aspergillus* spp. *Phytopathology* 84:483-488.
98. Sarr, A.B., K. Mayura, and T.D. Phillips. 1994. Effects of hydrated sodium calcium aluminosilicate on the metabolic profile of AFB₁ in Fischer-344 rats. *Toxicol. Lett.* 75:145-151.
99. Zhao, F., K. Mayura, R.W. Hutchinson, R.P. Lewis, R.C. Burghardt and T.D. Phillips. 1995. Developmental toxicity and structure-activity relationships of chlorophenols using human embryonic palatal mesenchymal cells. *Tox. Lett.* 78:35-42.
100. Phillips, T.D., A.B. Sarr, and P.G. Grant. 1995. Selective chemisorption and detoxification of aflatoxins by phyllosilicate clay. *Natural Toxins.* 3:204-213.
101. Washburn, K.S. and T.D. Phillips. 1995. Development of a field-practical assay for water-solvated chlorophenols. *J. Hazard. Mat.* 41:371-381.
102. Abo-Norag, M., T.S. Edrington, L.F. Kubena, R. B. Harvey, and T.D. Phillips. 1995. Influence of hydrated sodium calcium aluminosilicate and virginiamycin on aflatoxicosis in broiler chicks. *Poult. Sci.* 74:626-632.
103. Safe, S., K. Washburn, T. Zacharewski and T. Phillips. 1995. Synthesis and characterization of hydroxylated polychlorinated biphenyls (PCBs) identified in human serum. *Chemosphere.* 31:3017-3023.
104. Barhoumi, R., Mouneimne, Y., Phillips, T.D., Safe, S.H., and R.C. Burghardt. 1996. Alteration of oxytocin-induced calcium oscillations in clone 9 cells by toxin exposure. *Fund. Appl. Toxicol.* 33:220-228.
105. Zhao, F., Mayura, K., Kocurek, N., Edwards, J.F., Kubena, L.F., Safe, S.H. and T.D. Phillips. 1996. Inhibition of 3,3',4,4',5-Pentachlorobiphenyl-induced chicken embryotoxicity by 2,2',4,4',5,5'-Hexachlorobiphenyl. *Fund. Appl. Toxicol.* 35:1-8.
106. Zhao, F., Mayura, K., Harper, N., Safe, S.H. and T.D. Phillips. 1997. Inhibition of 3,3',4,4',5-Pentachlorobiphenyl induced fetal cleft palate and immunotoxicity in C57BL/6 mice by 2,2',4,4',5,5'-Hexachlorobiphenyl. *Chemosphere* 34:1605-1613.

107. Kubena, L.F., Edrington, T.S., Harvey, R.B., Phillips, T.D., Sarr, A.B., and G.E. Rottinghaus. 1997. Individual and combined effects of fumonisin B₁ present in *Fusarium moniliforme* culture material and diacetoxyscirpenol or ochratoxin A in turkey poults. *Poult. Sci.* 76:256-264.
108. Ramu, J., Clark, K., Woode, G.N., Sarr, A.B. and T.D. Phillips. 1997. Adsorption of cholera and heat-labile *Escherichia coli* enterotoxins by various adsorbents: An *in vitro* study. *J. Fd. Protect.* 60:1-5.
109. Dwyer, M.R., L.F. Kubena, R.B. Harvey, K. Mayura, A.B. Sarr, S. Buckley, R.H. Bailey, and T.D. Phillips. 1997. Effects of inorganic adsorbents and cyclopiazonic acid in broiler chickens. *Poult. Sci.* 76:1141-1149.
110. McKenzie, K.S., A. B. Sarr, K. Mayura, R.H. Bailey, D.R. Miller, T.D. Rogers, W.P. Norred, K.A. Voss, R.D. Plattner, L.F. Kubena, and T.D. Phillips. 1997. Oxidative degradation and detoxification of mycotoxins using a novel source of ozone. *Fd. Chem. Toxicol.* 35:807-820.
111. Kubena, L.F., T.S. Edrington, R.B. Harvey, S.A. Buckley, T.D. Phillips, G.E. Rottinghaus, and H.H. Caspers. 1997. Individual and combined effects of fumonisin B₁ present in *Fusarium moniliforme* culture material and T-2 toxin or deoxynivalenol in broiler chicks. *Poult. Sci.* 76:1239-1247.
112. Mayura, K., M.A. Abdel-Wahhab, K.S. McKenzie, A.B. Sarr, J.F. Edwards, K. Naguib, and T.D. Phillips. 1998. Prevention of maternal and developmental toxicity in rats via dietary inclusion of aflatoxin sorbents: Hidden risks. *Toxicol. Sci.* 41:175-182.
113. Grant, P.G., and T.D. Phillips. 1998. Isothermal adsorption of aflatoxin B₁ on HSCAS clay. *J. Ag. Fd. Chem.* 46:599-605.
114. Clark, K.J., A.B. Sarr, P.G. Grant, T.D. Phillips and G.N. Woode. 1998. In vitro studies on the use of clay, clay minerals and charcoal to adsorb bovine rotavirus and bovine coronavirus. *Vet. Microbiol.* 63:137-146.
115. Clark, K.J., P.G. Grant, A.B. Sarr, J.R. Belakere, C.L. Swaggerty, T.D. Phillips, and G.N. Woode. 1998. An in vitro study of theaflavins extracted from black tea to neutralize bovine rotavirus and bovine coronavirus infections. *Vet. Microbiol.* 63:147-157.
116. McKenzie, K.S., L.F. Kubena, A.J. Denvir, T.D. Rogers, G.D. Hitchens, R.H. Bailey, R.B. Harvey, S.A. Buckley, and T.D. Phillips. 1998. Aflatoxicosis in turkey poults is prevented by treatment of naturally-contaminated corn with ozone generated by electrolysis. *Poult. Sci.* 77:1094-1102.
117. Grant, P.G., S.L. Lemke, M.R. Dwyer and T.D. Phillips. 1998. Modified Langmuir equation for S-shaped and multisite isotherm plots. *J. Langmuir* 14(15):4292-4299.
118. Lemke, S.L., P.G. Grant and T.D. Phillips. 1998. Adsorption of zearalenone by organophilic montmorillonite clay. *J. Ag. Fd. Chem.* 46:3789-3796.

119. Mayura, K., H.J. Huebner, M.R. Dwyer, K.S. McKenzie, K.C. Donnelly, L.F. Kubena and T.D. Phillips. 1999. Multi-bioassay approach for assessing the potency of complex mixtures of polycyclic aromatic hydrocarbons. *Chemosphere* 38:1721-1732.
120. Huebner, H.J., Lemke, S.L., Ottinger, S.E., Mayura, K., and Phillips, T.D. (1999). Molecular characterization of high affinity, high capacity clays for the equilibrium sorption of ergotamine. *Food Additives and Contam.* 16:159-171.
121. Lemke, S.L., Mayura, K., Ottinger, S.E., McKenzie, K.S., Wang, N., Fickey, C., Kubena, L.F. and Phillips, T.D. 1999. Assessment of the estrogenic effects of zearalenone after treatment with ozone utilizing the mouse uterine weight bioassay. *J. Toxicol. Environ. Hlth.* 56:283-295.
122. Springman, K.R., K. Mayura, T. McDonald, K.C. Donnelly, L.F. Kubena and T.D. Phillips. 1999. Organoclay adsorption of wood-preserving waste from groundwater: Analytical and toxicological evaluations. *Toxicol. Environ. Chem.* 71(1-2):247-259.
123. Ottinger, S.E., Mayura, K., Lemke, S.L., McKenzie, K.S., Wang, N., Kubena, L.F., and Phillips, T.D. 1999. Utilization of electrochemically generated ozone in the degradation and detoxification of benzo[a]pyrene. *Toxicol. Environ. Hlth.* 56:565-583.
123. Phillips, T.D. 1999. Dietary clay in the chemoprevention of aflatoxin-induced disease. *Toxicological Sciences* 52:118-126.
124. Huebner, H.J., Mayura, K., Pallaroni, L., Ake, C.L., Lemke, S.L. and Phillips, T.D. 2000. Development and characterization of a carbon-based composite for decreasing patulin levels in apple juice. *J. Food Protection* 63:106-110.
125. Herrera, P., Burghardt, R.C. and Phillips, T.D. 2000. Adsorption of *Salmonella enteritidis* by cetyl pyridinium exchanged montmorillonite clays. *Vet. Microbiol.* (In press).

000413

ABSTRACTS AND PROCEEDINGS:

1. Phillips, T.D. and A.W. Hayes: 1977. Inhibition of *in vitro* adenosine triphosphatase activities in the mouse by patulin. Fed. Proc. 36:1008 (Abstract).
2. Desai, D., T.D. Phillips, A.W. Hayes, and I.K. Ho: 1978. Effects of aflatoxins on ATPase activities in rat and mouse tissues. Fed. Proc. 37:501 (Abstract).
3. Phillips, T.D., A.W. Hayes, I.K. Ho and D. Desai: 1978. Effects of rubratoxin B on the kinetics of cationic and substrate activation of Na⁺-K⁺ATPase and para nitrophenyl phosphatase activities. Fed. Proc. 37:502 (Abstract).
4. Hanna, G.D., T.D. Phillips, S.J. Cysewski, L.F. Kubena, G.W. Ivie, N.D. Heidelbaugh, D.A. Witzel, A.W. Hayes and W.L. Williams: 1980. High pressure liquid chromatographic determination of penicillic acid residues in poultry. Fed. Proc. 39(3,II):1102 (Abstract).
5. Phillips, T.D., B.R. Nechay, L.F. Kubena, S.J. Cysewski, A.W. Hayes and D.A. Witzel: 1981. High pressure liquid and gas-liquid chromatographic analysis of the diazomethane derivative of penicillic acid. Toxicologist (1):1:90 (Abstract).
6. Phillips, T.D., B.R. Nechay, L.F. Kubena, N.D. Heidelbaugh, E.C. Shepherd, A.F. Stein, S.L. Neldon and D.A. Witzel: 1981. Effects of calcium orthovanadate on Na⁺-K⁺ adenosinetriphosphatase activities in the chicken. Toxicologist (1):1:119 (Abstract).
7. Phillips, T.D., A.F. Stein, G.W. Ivie, N.D. Heidelbaugh and A.W. Hayes: 1981. High performance liquid chromatographic analysis of a diazomethane reaction product of ochratoxin A and its application to tissue detection and confirmation. Pharmacologist 23(3):115 (Abstract).
8. Kubena, L.F., T.D. Phillips, C.R. Creger, D.A. Witzel, and N.D. Heidelbaugh: 1982. Effect of ochratoxin A and tannic-Acid on various parameters in the growing chick. Poult. Sci. 61(7):1495 (Abstract).
9. Halvorson, M.R., L. Robertson, S.H. Safe and T.D. Phillips: 1983. Metabolism of aflatoxin B1 in rat microsomes: PCB congeners. Toxicologist 3(1):7 (Abstract).
10. Kubena, L.F., R.B. Harvey, S.L. Lovering, and T.D. Phillips: 1983. A chick model for impaired renal function. Poult. Sci. 62(7):1451 (Abstract).
11. Mayura, K., Berndt, W.O. and T.D. Phillips: 1983. Effect of simultaneous prenatal exposure to ochratoxin A and citrinin in rats. Fed. Proc. 42:624 (Abstract).
12. Shepherd, E.C., L.W. Robertson, N.D. Heidelbaugh, S.H. Safe and T.D. Phillips: 1983. Polyhalogenated biphenyls enhance formation and secretion of aflatoxin M1 in milk. Toxicologist 3(1):6 (Abstract).
13. Stein, A.F., W.O. Berndt and T.D. Phillips: 1984. Ochratoxin A-induced nephrotoxicity: comparative studies in the partially nephrectomized rat. Toxicologist 4(1):31 (Abstract).

14. Stein, A.F., R.G. Morgan, S. Geerling, H.H. Mollenhauer, N.D. Heidelbaugh, and T.D. Phillips: 1983. The effects of ochratoxin A in the partially nephrectomized rat. *Toxicologist* 3(1):149 (Abstract).
15. Walker, M.D. and T.D. Phillips: 1983. Effects of vanadate and vanadyl on sodium transport in toad urinary bladder. *Toxicologist* 3(1):75 (Abstract).
16. Wilczynski, T.A., N.D. Heidelbaugh, A.W. Hayes and T.D. Phillips: 1983. Effects of the mycotoxin penicillic acid on electrogenic sodium transport. *Toxicologist*. 3(1):10 (Abstract).
17. Halvorson, M.R., T.R. Irvin and T.D. Phillips: 1984. Analysis of aflatoxin B1 and metabolites via reverse phase high performance liquid chromatography. *Fed. Proc.* 43(3):578 (Abstract).
18. Harvey, R.B., L.F. Kubena, O.J. Fletcher, T.D. Phillips and L.D. Rowe: 1984. The toxicity of deoxynivalenol (vomitoxin) contaminated wheat to growing chicks. *J. Am. Vet. Med. Assoc.* 185:339 (Abstract).
19. Kubena, L.F., R.B. Harvey, T.D. Phillips and O.J. Fletcher: 1984. Effects of ochratoxin A and vanadium on various parameters in growing chicks. *Poult. Sci.* 63:132 (Abstract).
20. Mayura, K., W.O. Berndt and T.D. Phillips: 1984. Effects of phenylalanine on the teratogenicity of ochratoxin A in rats. *Toxicologist* 4(1):165 (Abstract).
21. Parker, R.W., L.F. Kubena, N.D. Heidelbaugh and T.D. Phillips: 1984. The Effects of dietary phenylalanine supplementation on ochratoxicosis in normal and partially nephrectomized rats. *Toxicologist*. 4(1):11 (Abstract).
22. Shepherd, E.C. and T.D. Phillips: 1984. A micro-column method for aflatoxin detection. *Toxicologist* 4(1):128 (Abstract).
23. Sylvia, V.L., T.D. Phillips, T.R. Irvin and N.D. Heidelbaugh: 1984. Analysis of deoxynivalenol by high pressure liquid chromatography with electrochemical detection. *Toxicologist* 4(1):50 (Abstract)
24. Clement, B.A., and T.D. Phillips: 1985. Advances in the detection and determination of mycotoxins via capillary GC/quadrupole mass spectrometry. *Toxicologist* 5(1):232. (Abstract)
25. Green, J.L., V.L. Sylvia, N.D. Heidelbaugh and T.D. Phillips: 1985. Biosynthesis of radiolabeled deoxynivalenol by Fusarium roseum in liquid culture. *Toxicologist* 5(1):234. (Abstract)
26. Huff, W., L. Kubena, R. Harvey, T. Phillips and C. Creger: 1985. Aflatoxin and deoxynivalenol interaction in broilers. *Poult. Sci.* 64:131. (Abstract)
27. Sylvia, V.L., J.L. Green, N.D. Heidelbaugh and T.D. Phillips: 1985. Application of an electrochemical detection method for the analysis of DON. *Toxicologist*. 5(1):232 (Abstract).

28. Kubena, L.F., R.B. Harvey, and T.D. Phillips: 1985. Effects of feeding DON contaminated wheat to mature laying hens. *Poult. Sci.* 64:131 (Abstract).
29. Mayura, K., E.E. Smith, N.D. Heidelbaugh and T.D. Phillips: 1985. DAS induced prenatal dysmorphogenesis in the mouse. *Toxicologist* 5(1):187. (Abstract).
30. Harvey, R.B., W.E. Huff, L.F. Kubena, D.E. Corrier, D.E. Clark and T.D. Phillips: 1986. Effects of feeding diets contaminated with aflatoxin and deoxynivalenol to growing pigs. *Proc. Am. Assoc. Vet. Lab. Diagn* (p. 21).
31. Harvey, R.B., L.F. Kubena, W.E. Huff, D.E. Corrier, D.E. Clark and T.D. Phillips: 1986. Aflatoxicosis of growing pigs - a pathophysiologic study. *J. Am. Vet. Med. Assoc.* 189(3):339 (Abstract).
32. Harvey, R.B., L.F. Kubena, T.D. Phillips, and N.D. Heidelbaugh: 1986. Validation of impaired renal function chick model with uranyl nitrate. 64:131 (Abstract).
33. Kubena, L.F., W.E. Huff, R.B. Harvey, D.E. Corrier, T.D. Phillips and C.R. Creger: 1986. Influence of ochratoxin A (OA) and deoxynivalenol (DON) on growing broiler chicks. *Poult. Sci.* 65:73.
34. Kubena, L.F. and T.D. Phillips: 1986. Toxicology of vanadium. Symposium summary: Role of vanadium in biology. *Proc. Fed. Am. Soc. Exp. Biol.* 45(2):128 (Abstract).
35. Davidson, J.N., J.G. Babish, K.A. Delaney, D.R. Taylor and T.D. Phillips: 1987. Hydrated sodium calcium aluminosilicate decreases the bioavailability of aflatoxin in the chicken. *Poult. Sci.* 66(1):89 (Abstract).
36. Haake, J.M., K. Mayura, T.D. Phillips, and S. Safe: 1987. Teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin: Antagonism by arochlor 1254. *Toxicologist* 7(1):124 (Abstract).
37. Halvorson, M.R., S.H. Safe, A. Parkinson, and T.D. Phillips: 1987. Aflatoxin B1 hydroxylation by the PCN-inducible form of rat liver microsomal cytochrome P-450. *Toxicologist* 7:219 (Abstract).
38. Kubena, L.F., W.E. Huff, R.B. Harvey, D.E. Corrier, T.D. Phillips and G.E. Rottinghaus: 1987. Influence of ochratoxin A (OA) and T-2 toxin on broiler chicks. *Poult. Sci.* 66:130 (Abstract).
39. Maull, E.A., B.A. Clement, N.D. Heidelbaugh and T.D. Phillips: 1987. Effects of aflatoxins B1 and G1 on postimplantation rat embryos. *Toxicologist* 7:175 (Abstract).
40. Phillips, T.D., L.F. Kubena, R.B. Harvey, D.R. Taylor, and N.D. Heidelbaugh: 1987. Mycotoxin hazards in agriculture: New approach to control. *J. Am. Vet. Med. Assoc.* 190(12):1617 (Abstract).
41. Harvey, R.B., L.F. Kubena, T.D. Phillips, and W.E. Huff: 1988. Possible methods to combat the mycotoxin problem. *Plains Nutr. Council Symp. Mycotoxins in Livestock Feeds.* pp. 1E-4E. (Proceedings).

42. Harvey, R.B., L.F. Kubena, T.D. Phillips, W.E. Huff and D.E. Corrier: 1988. Approaches to the prevention of aflatoxicosis. Proc. MD Nutr. Conf. Feed Manufacturers. pp. 102-107: (Proceedings).
43. Harvey, R.B., L.F. Kubena, T.D. Phillips, W.E. Huff, and D.E. Corrier: 1988: Prevention of clinical signs of aflatoxicosis with hydrated sodium calcium aluminosilicate added to diets. Proc. Am. Assoc. Swine Prac. p. 99-102.
44. Harvey, R.B., T.D. Phillips, L.F. Kubena, and W.E. Huff: 1988. Dietary Hydrated Sodium calcium aluminosilicate and its impact on aflatoxin toxicity in pigs and milk residues in dairy cows. Proc. Am. Assoc. Vet. Lab. Diagn. p. 67. (Abstract)
45. Kubena, L.F., R.B. Harvey, T.D. Phillips and N.D. Heidelbaugh: 1988. Novel approach to the preventive management of aflatoxicosis in poultry. U.S. Animal Health Assoc. pp.302-304.
46. Kubena, L.F., R.B. Harvey, T.D. Phillips and W.E. Huff: 1988. Modulation of aflatoxicosis in growing chickens by dietary addition of a hydrated sodium calcium aluminosilicate. Poultry Sci. 67:106 (Abstract).
47. Phillips, T.D.: June 1988. Selectively Adsorbed Mycotoxins (SAM); The Texas A&M University System, Texas Agricultural Experiment Station, Focus on the Future Research Report, MP-1625, Processing Technology.
48. Harvey, R.B., L.F. Kubena, M.H. Elissalde, P.S. Holt, W.E. Huff, and T.D. Phillips: 1989. Evaluation of humoral and cell-mediated immune status of chicks fed deoxynivalenol-contaminated wheat diets. Proc. IX Intl. Congress World Vet. Poultry Assoc., Brighton, England. p. 121. (Proceedings)
49. Harvey, R.B., L.F. Kubena, T.D. Phillips, and W.E. Huff: 1989. Mycotoxins--danger, prevention and control. Latin America Poult. Health Symp., Guanacaste, Costa Rica, Nov. 10-11, 1989. pp. 1-14. (Proceedings)
50. Huff, W.E., L.F. Kubena, R.B. Harvey, and T.D. Phillips: 1989. Efficacy of hydrated sodium calcium aluminosilicate to reduce the combined toxicity of aflatoxin and ochratoxin A. Poultry Sci. 68(Suppl. 1):69. (Abstract)
51. Kubena, L.F., R.B. Harvey, W.E. Huff, D.E. Corrier, and T.D. Phillips: 1989. Ameliorating properties of a hydrated sodium calcium aluminosilicate on the toxicity of aflatoxin and T-2 toxin. Poultry Sci. 68:(Suppl. 1):81. (Abstract)
52. Mayura, K., J.F. Edwards, E.A. Maull and T.D. Phillips: 1989. The effects of ochratoxin A on postimplantation rat embryos in culture. Arch. Environ. Contam. Toxicol. 18:411-415 (Abstract).
53. Bailey, R.H., B.A. Clement, J.M. Phillips, A.B. Sarr, T.A. Turner and T.D. Phillips: 1990. Fate of aflatoxins in lime processed corn. Toxicologist 10(1):163 (Abstract).
54. Bean, M.S., K. Mayura, B.A. Clement, J.F. Edwards, R.B. Harvey and T.D. Phillips: 1990. Studies of prenatal development in the rat following oral exposure to T-2 toxin. Toxicologist 10(1):124 (Abstract).

55. Clement, B.A., A.B. Sarr, K. Mayura and T.D. Phillips: 1990. Selective adsorption of mycotoxins (SAM): Field-practical method of multi-mycotoxin detection. *Toxicologist* 10(1):340 (Abstract).
56. Ellis, J.A., R.B. Harvey, L.F. Kubena, R.H. Bailey, B.A. Clement and T.D. Phillips: 1990. Reduction of aflatoxin M1 residues in milk utilizing hydrated sodium calcium aluminosilicate. *Toxicologist* 10(1):163 (Abstract).
57. Kubena, L., W. Huff, R. Harvey, A. Yersin, M. Elissalde, D. Witzel, L. Giroir and T.D. Phillips: 1990. Effects of a hydrated sodium calcium aluminosilicate on growing turkey poults during aflatoxicosis. *Poultry Sci.* 69(Suppl. 1): 175. (Abstract)
58. Mayura, K., E.E. Smith, B.A. Clement and T.D. Phillips: 1990. Detection and ranking of developmental hazards associated with chlorinated phenols. *Toxicologist* 10(1):273 (Abstract).
59. Phillips, T.D., B.A. Clement, L.F. Kubena and R.B. Harvey: 1990. Prevention of aflatoxicosis in animals and aflatoxin residues in food of animal origin with hydrated sodium calcium aluminosilicate. *Proc. World Assoc. Vet. Food Hygienists: Xth Intl Symp., Stockholm, Sweden (July, 1989)*, pp.103-108.
60. Phillips, T.D., B.A. Clement, L.F. Kubena and R.B. Harvey: 1990. Use of dietary chemisorbents to prevent aflatoxicosis in farm animals. *Perspective on Aflatoxin in Field Crops and Animal Food Products in the United States, USDA/ARS-83*, pp. 106-114. *Natl Tech. Information Svc Symp., Springfield, VA. Jan. 23-24. 1990.* (Proceedings)
61. Phillips, T.D., B.A. Clement, L.F. Kubena and R.B. Harvey: 1990. Selective chemisorption of aflatoxin by hydrated sodium calcium aluminosilicate: Prevention of aflatoxicosis in animals and reduction of aflatoxin residues in food of animal origin. *NC-151/NC-129 Symposium on Aflatoxin in Corn, Kansas City, Missouri.*
62. Richard, J.L., R.J. Cole, T.D. Phillips, et al.: 1990. *Mycotoxins: economics and health risks. Council for Agricultural Science and Technology Task Force Report No. 116, CAST, Ames, Iowa.*
63. Sarr, A.B., B.A. Clement and T.D. Phillips: 1990. Effects of molecular structure on the chemisorption of aflatoxin B1 and related compounds by hydrated sodium calcium aluminosilicate. *Toxicologist* 10(1):163 (Abstract).
64. Yang, Y.G., K. Mayura and T.D. Phillips: 1990. Evaluation of the developmental toxicity of citrinin using *hydra attenuata* and postimplantation rat whole embryo culture. *Toxicologist* 10(1):273 (Abstract).
65. Harvey, R.B., L.F. Kubena, T.D. Phillips and W.E. Huff: 1991. Review of the protective effects of hydrated sodium calcium aluminosilicate (HSCAS) on the toxicity of aflatoxin to chicks and poults. *Proc. 40th Western Poult. Dis. Conf.* pp.115-117 (Proceedings).
66. Kubena, L., R. Harvey, T. Phillips, B. Clement and M. Elissalde: 1991. Effects of HSCAS on broiler chicks during aflatoxicosis. *Poult. Sci.* 70 (1):68 (Abstract).

67. Phillips, T.D., T.R. Narasimhan, K. Mayura, B.A. Clement, and S. Safe: 1991. Effect of chlorinated phenols on rat embryonic and hepatic mitochondrial oxidative phosphorylation. *Toxicologist* 11:71 (Abstract).
68. Ellis, J.A., R.H. Bailey, B.A. Clement and T.D. Phillips: 1991. Chemisorption of aflatoxin M₁ from milk by hydrated sodium calcium aluminosilicate. *Toxicologist* 11:96 (Abstract).
69. Sarr, A.B., B.A. Clement and T.D. Phillips: 1991. Molecular mechanism of aflatoxin B₁ chemisorption by hydrated sodium calcium aluminosilicate. *Toxicologist* 11:97 (Abstract).
70. Yang, Y.G., D.N. McMurray, B.A. Clement, J.P. Stack and T.D. Phillips: 1991. Immunotoxicity associated with a thermotolerant strain of *Penicillium citrinum*. *Toxicologist* 11:203 (Abstract).
71. Burghardt, R.C., R. Mouneimne, E.J. Lewis, R.H. Bailey and T.D. Phillips: 1991. Analysis of patulin toxicity with in vitro fluorescence assays. *Toxicologist* 11:275 (Abstract).
72. Bailey, R.H., A.B. Sarr, B.A. Clement, T.A. Chase and T.D. Phillips: 1991. The distribution of aflatoxins in the production of corn tortillas. *Toxicologist* 11:280 (Abstract).
73. Machen, M.D., K. Mayura, B.A. Clement and T.D. Phillips: 1991. Utilization of in vitro bioassays to assess the effectiveness of chemisorption of aflatoxin B₁ by hydrated sodium calcium aluminosilicate. *Toxicologist* 11:280 (Abstract).
74. Huang, Z.G., G-Y Hwang, B.A. Clement, R.H. Bailey, J.A. Ellis and T.D. Phillips: 1991. A field practical method for rapid detection and semi-quantitation of aflatoxin M₁ and sulfamethazine residues in milk. *Toxicologist* 11:280 (Abstract).
75. Dryden, C.J., K. Mayura, B.A. Clement, M. Becker and T.D. Phillips: 1991. Evaluation of the developmental toxicity of cyclopiazonic acid using *Hydra attenuata* and postimplantation rat whole embryo bioassays. *Toxicologist* 11:296 (Abstract).
76. Becker, M., K. Mayura, C.J. Dryden, B.A. Clement and T.D. Phillips: 1991. The effects of polychlorinated diphenyl ethers in cultures of adult hydra and postimplantation rat embryos. *Toxicologist* 11:296 (Abstract).
77. Small, M., E. Smith, C. Braithwaite, E. Duffus, T. Phillips and A. Reine: 1991. Evaluation of the developmental toxicity of ochratoxin A and cyclopiazonic acid in postimplantation rat embryos. *Toxicologist* 11, 297 (Abstract).
78. Mayura, K., W.S. Conover, B.A. Clement and T.D. Phillips: 1991. Comparison of the developmental toxicities of common penicillium mycotoxins using the hydra bioassay. *Toxicologist* 11, 297 (Abstract).
79. Smith, E., C. Braithwaite, M. Small, A. Reine and T.D. Phillips: 1991. Alteration in protein synthesis induced by ochratoxin A in postimplantation rat whole embryo culture. *Toxicologist* 11, 297 (Abstract).

80. Mayura, K., B.A. Clement, S. Safe and T.D. Phillips: 1992. Teratogenic effects of 3,3',4,4',5-pentachlorobiphenyl in C57BL/6 mice. *Toxicologist* 12, 104 (Abstract).
81. Hwang, G-Y, B.A. Clement, Z. Huang, K. Mayura and T.D. Phillips: 1992. Rapid field-practical diagnostic assays for the detection of fumonisin B1, cyclopiazonic acid, deoxynivalenol, aflatoxin and mixtures of these mycotoxins. *Toxicologist* 12, 261 (Abstract).
82. Smith, E.E., C.E. Braithwaite, M.H. Small, T.D. Phillips and A.H. Reine: 1992. Changes in HSP 70 in ochratoxin A treated embryos *in vitro*. *Toxicologist* 12, 332 (Abstract).
83. Small, M. H., E.E. Smith, C.E. Braithwaite, T.D. Phillips and A.H. Reine: 1992. Effects of patulin on postimplantation rat embryos. *Toxicologist* 12, 334 (Abstract).
84. Burghardt, R.C., R. Barhoumi, R.H. Bailey, B.A. Clement and T.D. Phillips: 1992. Analysis of cellular toxicity using *in vitro* fluorescence assays. *Toxicologist* 12, 373 (Abstract).
85. Phillips, T.D., B.A. Clement, A.B. Sarr, R.B. Harvey and L.F. Kubena: 1992. A practical approach to the control of aflatoxins: Selective chemisorption by phyllosilicate clays. *Proceedings of the 3rd World Congress on Foodborne Infections and Intoxications, Berlin, Germany*, pp. 687-689.
86. Clement, B.A., T.D. Phillips and Z. Hwang: 1992. Chemiselective immobilization and detection of mycotoxins: A field-practical approach to detection. *Proceedings of the 3rd World Congress on Foodborne Infections and Intoxications, Berlin, Germany*, pp. 653-656.
87. Gentles, A.B., M.H. Small, E.E. Smith, T.D. Phillips, E. Duffus, C.E. Braithwaite: 1993. Teratogenic effects of orally administered diacetoxyscirpenol in mice. *Toxicologist* 13:79.
88. Huang, Z.G., A.B. Sarr, A.L. Fisher, M.R. Dwyer, S.M. Williams, B.A. Clement and T.D. Phillips. 1993. A rapid method for the detection of fumonisin in corn. *Toxicologist* 13:145.
89. Cathey, C.G., Z.G. Huang, A.B. Sarr, M.R. Dwyer, B.A. Clement and T.D. Phillips: 1993. A sensitive minicolumn method for the detection of aflatoxin M₁ in whole milk. *Toxicologist* 13:145.
90. Mayura, K., Y.G. Yang, B. Spainhour, J.F. Edwards and T.D. Phillips: 1993. Developmental toxicity evaluation of ochratoxin A and citrinin in combination utilizing the *Hydra attenuata* and rat whole embryo culture bioassays. *Toxicologist* 13:257.
91. Johnson, P., E.E. Smith, T.D. Phillips, A.B. Gentles, M. Small, E. Duffus. The effects of fumonisin B₁ on rat embryos in culture. *Toxicologist* 13:257.
92. Spainhour, B., K. Mayura, f. Zhao and T.D. Phillips: 1993. Evaluation of the developmental toxicity of 3,5-dichlorophenol. *Toxicologist* 13:258.

93. White, P., A.B. Sarr, K. Mayura, B. Spainhour and T.D. Phillips: 1993. Binding of secalononic acid D to hydrated sodium calcium aluminosilicate. *Toxicologist* 13:258.
94. Washburn, K.S., B. Clement, M. Connolly, M. Harmon, and T.D. Phillips: 1993. A field-practical assay for environmental toxins: A rapid detection method for chlorophenols. *Toxicologist* 13:268.
95. Bailey, R.H., R. Barhoumi, R.C. Burghardt and T.D. Phillips: 1993. Fluorescence assessment of ochratoxin A-induced cellular toxicity. *Toxicologist* 13:452.
96. Phillips, T.D.: 1993. Selective chemisorption of aflatoxin by phyllosilicate clay. Proceedings of the 11th International Conference on Aviculture, Mexico City, Mexico, pp. 147-166.
97. Barhoumi, R., R.H. Bailey, R. Hutchinson, J.E. Echols and T.D. Phillips. 1994. Octanol enhances toxicity of melphalan and reversal of drug resistance in an ovarian adenocarcinoma line. *Toxicologist* 14:58.
98. Spainhour, C.B., R. Barhoumi, K. Mayura, R. Burghardt and T.D. Phillips. 1994. Laser cytometric analyses of select chlorophenol congeners in clone 9 hepatocytes. *Toxicologist* 14:58.
99. Hutchinson, R.W., C.B. Spainhour, R.H. Bailey, T.D. Phillips, J.A. Bowen, R. Barhoumi and R.C. Burghardt. Kinetic analysis of rhodamine 123 fluorescence reveals a triphasic pattern following treatment with several toxins. *Toxicologist* 14:100.
100. Zhao, F., K. Mayura, R.W. Hutchinson, R.P. Lewis, R.C. Burghardt and T.D. Phillips. 1994. Human embryonic palatal mesenchymal (HEPM) cell growth inhibition bioassay for evaluation of the chlorophenols. *Toxicologist* 14:102.
101. Sarr, A., K. Mayura and T. Phillips. 1994. Effects of HSCAS on the metabolic profile of AFB₁ in Fischer-344 rats. *Toxicologist* 14:208.
102. Bailey, R.H., R. Barhoumi, R.C. Burghardt, R. Lewis and T.D. Phillips. 1994. Fluorescence analysis of patulin and ochratoxin A induced cytotoxicity. *Toxicologist* 14:209.
103. White, P., A. Sarr, K. Mayura, P. Grant, K. Washburn, M. Dwyer, J. Ellis and T. Phillips. 1994. Determination of the binding ability of different sorbents for secalononic acid D. *Toxicologist* 14. 212.
104. Dwyer, M.R., A.B. Sarr, K. Mayura, K.S. Washburn and T.D. Phillips. 1994. Evaluation of the binding efficacy of selected sorbents for cyclopiazonic acid. *Toxicologist* 14:212.
105. Grant, P.G., A.B. Sarr, R.H. Bailey, J.R. Snell and T.D. Phillips. 1995. Characterization of hydrated sodium calcium aluminosilicate and aflatoxin B₁ binding. *Toxicologist* 14:74.
106. Mayura K., M.A. Abdel-Wahhab, A.B. Sarr, M.L. Cavazos-Martinez, J.F. Edwards, K. Naguib, S.O. Amin, and T.D. Phillips. 1995. Selective chemisorption of aflatoxin B₁ by HSCAS: Prevention of developmental toxicity in the rat. *Toxicologist* 14:74.

107. Dwyer, M.R., A.B. Sarr, K. Mayura, K.S. Washburn, and T.D. Phillips. 1995. A clay-based method for the adsorption and inactivation of cyclopiazonic acid. *Toxicologist* 14:74.
108. Herrera, P., A.B. Sarr, J. Ramu, and G.N. Woode. 1995. Investigation of cholera toxin adsorption by phyllosilicate clays. *Toxicologist* 14:75-75.
109. Zhao, F., K. Mayura, S. Safe and T.D. Phillips. 1995. 2,2',4,4',5,5'-hexachlorobiphenyl as an antagonist of the teratogenicity of 3,3',4,4',5-pentachlorobiphenyl in C57BL/6 mice. *Toxicologist* 14:157.
110. Washburn, K. and T. Phillips. 1995. Dechlorination of pentachlorophenol by chemically modified montmorillonite clays. *Toxicologist* 14:170.
111. McKenzie, K.S., A.B. Sarr, R.H. Bailey, D.R. Miller, L. Kubena and T.D. Phillips. 1995. Oxidative degradation of aflatoxins using a novel method of ozone production. *Toxicologist* 14: 215.
112. White, P., A. Sarr, K. Mayura, H. Bailey, P. Grant, and T. Phillips. 1995. Investigation of the adsorption of SAD to phyllosilicate clay. *Toxicologist* 14:216.
113. Barhoumi, R., T.D. Phillips, and R.C. Burghardt. 1995. Analysis of the chronology of patulin-induced cellular injury: Multiparameter fluorescence kinetic analysis. *Toxicologist* 14:282.
114. Barhoumi, R., T.D. Phillips and R.C. Burghardt. 1996. Comparative analysis of the chronology of patulin and gossypol-induced cellular injury: Multiparameter fluorescence kinetic analysis. *Toxicologist* 30:126.
115. Burghardt, R.C., T.D. Phillips, S.H. Safe and R. Barhoumi. 1996. Alteration of oxytoxin-induced calcium oscillations in clone 9 cells by toxin exposure. *Toxicologist* 30:128.
116. Zhao, F., K. Mayura, N. Kocurek, J.F. Edwards, L.F. Kubena, S. Safe and T.D. Phillips. 1996. Effects of 2,2',4,4',5,5'-hexachlorobiphenyl and indole-3-carbinole on 3,3',4,4',5-pentachlorobiphenyl-induced teratogenesis in chicken embryos and C57BL/6 mice. *Toxicologist* 30:197.
117. Bailey, R.H., A.B. Sarr and T.D. Phillips. 1996. Alkaline treatment of aflatoxin B₁: Stability of parent and degradation products. *Toxicologist* 30:212.
118. Grant, P., A.B. Sarr and T.D. Phillips. 1996. Isothermal analysis of aflatoxin B₁ binding to phyllosilicate clay. *Toxicologist* 30:212.
119. Dwyer, M.R., K. Mayura, A.B. Sarr, L.F. Kubena, S. Buckley, R.B. Harvey, R.H. Bailey and T.D. Phillips. 1996. Effects of clay and cyclopiazonic acid in broiler chickens. *Toxicologist* 30:213.
120. McKenzie, K., A. Sarr, K. Mayura, W. Norred, R. Plattner, T. Rogers and T. Phillips. 1996. Degradation and toxicological evaluation of fumonisin B₁ and other mycotoxins treated with ozone gas. *Toxicologist* 30:213.

121. Zhao, F., Mayura, K., Edwards, J.F., Kubena, L., Safe, S. and T.D. Phillips. 1996. Effects of indole-3-carbinol on 3,3,4,4,5-Pentachlorobiphenyl induced teratogenesis in chicken embryos and in C57BL/6 mice. Proceedings of the Annual Conference on Dioxins, Amsterdam, the Netherlands.
122. McKenzie, S., Denvir, A.J., Rogers, T.D., Kubena, L.F., Mayura, K., Dwyer, M.R. and T.D. Phillips. 1997. Toxicity and degradation of aflatoxin B₁ in field-contaminated corn treated with electrolytically-generated ozone gas. Toxicologist 36:40.
123. Grant, P.G. and T.D. Phillips. 1997. Isothermal analysis and thermodynamic measurement of aflatoxin B₁ binding to phyllosilicate clays. Toxicologist 36:41.
124. Herrera, P., Springman, K. and T.D. Phillips. 1997. Adsorption of *Salmonella enteritidis* by cetyl pyridinium exchanged phyllosilicate clays. Toxicologist 36:42.
125. Mayura, K., Zhao, F., Dwyer, M.R., McKenzie, K.S., Washburn, K.S., Bailey, R.H., Donnelly, K.C., Kubena, L.F. and T.D. Phillips. 1997. Evaluation of the toxicity of polycyclic aromatic hydrocarbons from coal tar utilizing the chick embryotoxicity screening test (CHEST). Toxicologist 36:219.
126. Springman, K.R., Grant, P.G., Washburn, K.S. and T.D. Phillips. 1997. Adsorption of pentachlorophenol by cetyl pyridinium-modified acidic clay. Toxicologist 36:282.
127. Lemke, S.L., P.G. Grant, and T.D. Phillips. 1997. Isothermal analysis of zearalenone on organo-substituted clays. 214th ACS Meeting, Las Vegas, Abstract # 031.
128. Grant, P.G., S.L. Lemke, M.R. Dwyer and T.D. Phillips. 1997. Modified Langmuir equation for S-shaped and multi-site isotherm plots. Proceedings of the 214th American Chemical Society Meeting, Las Vegas, Abstract # 024.
129. Ottinger, S.E., K. Mayura, K.S. McKenzie, L.F. Kubena and T.D. Phillips. 1998. Degradation of benzo[a]pyrene via electrochemically generated ozone and ozone/uv light. Toxicol. Sci. 42:31.
130. Lemke, S.L., K.S. McKenzie, P.G. Grant and T.D. Phillips. 1998. Fumonisin B₁ adsorption onto organo-substituted clays. Toxicol. Sci. 42:290.
131. Huebner, H., S. Lemke, S. Ottinger, P. Grant and T.D. Phillips. 1998. Equilibrium adsorption of ergotamine by various phyllosilicate clays. Toxicol. Sci. 42:290.
132. Herrera, P., P.G. Grant, K. Springman, R.C. Burghardt and T.D. Phillips. 1998. Quantification of the antibacterial properties of montmorillonites exchanged with cationic surfactants. Toxicol. Sci. 42:290.
133. Mouneimne, Y., R. Barhoumi, T.D. Phillips, and R.C. Burghardt. 1998. Alteration of calcium oscillations by elf electric field in clone 9 cells. Toxicol. Sci. 42:335.
134. Springman, K.R., T.J. McDonald, K. Mayura, K.C. Donnelly, and T.D. Phillips. 1998. Organoclay adsorption of components of a complex mixture from groundwater. Toxicol. Sci. 42:335.

135. Lemke, S.L., S.E. Ottinger, and T.D. Phillips. 1998. Sorption of fumonisin B₁ by nonexchanged and organo-substituted clays. American Chemical Society Meeting, Boston, MA.
136. Ottinger, S.E., K. Mayura, S. McKenzie, S. Lemke, L. Kubena and T.D. Phillips. 1998. Degradation and detoxification of benzo[a]pyrene following treatment with ozone. American Chemical Society Meeting, Boston, MA.
137. Barhoumi, R., K. Ramos, S.H. Safe, T.D. Phillips, and R.C. Burghardt. 1999. Analysis of the subcellular distribution and cytotoxicity of benzo[a]pyrene in rat liver cells. *Toxicol. Sci.* 48:15.
138. Huebner, H.J., K. Mayura, C.L. Ake, S.L. Lemke and T.D. Phillips. 1999. Application of immobilized activated carbon for the sorption of patulin from aqueous solution. *Toxicol. Sci.* 48:55.
139. Mouneimne, Y, R. Barhoumi, T.D. Phillips, S.H. Safe and R.C. Burghardt. 1999. Alteration of oxytocin- and vasopressin-induced calcium oscillations in a rat liver cell line by TCDD. *Toxicol. Sci.* 48:64.
140. Ake, C.L., K. Mayura, H.J. Huebner, G.R. Bratton, and T.D. Phillips. 1999. Equilibrium adsorption of lead in water by trioctahedral smectite clay. *Toxicol. Sci.* 48:329.

BOOK CHAPTERS:

1. Toom, P.M. and T.D. Phillips: 1978. Effects of purified components of jellyfish toxin on adenosine triphosphatase activities. In *Toxins, Animal, Plant and Microbial*, P. Rosenberg (Ed.), Pergamon Press, pp. 527-538.
2. Phillips, T.D., T. Fedorowski and A.W. Hayes: 1982. Techniques in membrane toxicology. In *Principles and Methods in Toxicology*, Raven Press, pp. 587-609.
3. Phillips, T.D. and A.W. Hayes: 1989. Techniques in membrane toxicology. In *Principles and Methods in Toxicology* (2nd. Edition). A.W. Hayes (Ed.), Raven Press.
4. Phillips, T.D., A. Sarr, B. Clement, L. Kubena and R. Harvey: 1991. Prevention of aflatoxicosis in farm animals via selective chemisorption of aflatoxin. In *Mycotoxins, Cancer, and Health* (Vol.1). G. Bray and D. Ryan, LSU, pp. 223-237.
5. Phillips, T.D., B.A. Clement, and D.L. Park: 1994. Approaches to reductions of aflatoxins in foods and feeds. In *The Toxicology of Aflatoxins: Human Health, Agricultural and Veterinary Significance*. Academic Press., pp. 383-405.
6. Burghardt R.C., R. Barhoumi, D. Doolittle, and T.D. Phillips: 1994. Application of cellular fluorescence imaging for *in vitro* toxicology testing. In: *Principles and Methods of Toxicology*, AW Hayes, Ed. Raven Press, New York, pp. 1231-1258.

PATENTS, DISCLOSURES AND LICENSE AGREEMENTS:

1. *Selective Immobilization and Detection of Mycotoxins in Solution*. 1992 U.S. Patent No. 5,178,832; Target™ technology licensed by the Texas A&M System to: TerraTek Inc., Salt Lake City, Utah.
2. *Method for Inactivating Mycotoxins Present in Animal Feed*. 1993 U.S. Patent No. 5,165,946. NovaSil™ technology licensed by the Texas A&M System to: Engelhard Chemicals, Cleveland, Ohio.
3. *Mycotoxin screening technology*. 1995 license to VICAM, Ltd, Watertown, MA.
4. Disclosure of invention: *Claypak affinity column for the rapid cleanup and identification of contaminated food, feed and biological samples* (TAMU Technology Licensing Office, 1995).
5. Disclosure of invention: *Dechlorination of water-solvated pentachlorophenol by ammonium-exchanged calcium montmorillonite* (TAMU Technology Licensing Office, 1995).
6. Disclosure of invention: *Equilibrium adsorption of zearalenone by organophilic montmorillonite clay* (TAMU Technology Licensing Office, 1997).
7. *Matrix-Immobilized adsorbents for the removal of chemically diverse contaminants from aqueous solutions*. *United States Patent Application No. 09/267,470* (TLO # 907), Filed 03/10/99.

SCIENTIFIC PRESENTATIONS (EXAMPLES):

Massachusetts Institute of Technology (MIT), Applied Biology and Chemistry
Frito-Lay, Applied and Basic Research Section
Department of Pharmacology and Toxicology, School of Medicine, TAMU
Department of Pharmacology and Toxicology, UTMB, Galveston, TX
Technical Conference of the IFT (Institute of Food Technologists)
Southern Regional Research Center, New Orleans, LA
TVMA convention, Houston, TX
Texas Pharmacology Meetings, University of Texas, Austin, TX
Department of Pharmacology and Toxicology, UT Medical School, Houston, TX
American Oil Chemists Society Meeting, Toronto, Canada
FASEB Meeting, ASPET Symposium, New Orleans, LA
Department of Food Science and Technology, Texas A&M University
Monsanto - Agrichemical Research and Development, St. Louis, MO
Kaiser Aluminum and Chemical Corp., Center for Technology, Oakland, CA
AOAC Spring Training Workshop, Dallas, TX
FASEB meeting, ASPET Symposium on Trace Metals, Anaheim, CA
Harshaw/Filtrol Corporation, Cleveland, OH
APRES Meeting, Chair, Mycotoxins Session, San Antonio, TX
Institute of Food Technologists - Short Course, Dallas, TX
Michigan State University, Lansing, MI
Kaiser Chemicals, Cleveland, OH

000425

NIH Toxicology Training Grant Symposium, VMI, Richmond, VA
American Veterinary Medical Association Meeting, Public Health Symposium, Chicago, IL
Kaiser Chemicals Symposium, Chicago, IL
Cornell University, Mycotoxin Presentation, Ithaca, N.Y.
CAST (Council for Agricultural Science and Technology) Meeting, Kansas City, MO
AVMA Sponsored Mycotoxin Symposium, Mycotoxins, Arlington, VA
Delmarva Poultry Meeting, Mycotoxins, Ocean City, Md
AMPI (Associated Milk Producers, Inc.) Meeting, Mycotoxins, Dallas, TX
Texas Department of Health (TDH) Meeting, Mycotoxins, Austin, TX
Office of the State Chemist, Mycotoxins, TAMU
Institut de Technologie Alimentaire, Dakar, Senegal, West Africa
Texas Corn Growers Association, Detoxification of Aflatoxins, Corpus, Christi
Congressional Symposium on Mycotoxins, Washington, D.C.
American Peanut Research and Education Society, Mycotoxins, North Carolina
World Congress on Veterinary Food Hygiene, Stockholm, Sweden
NC-151/NC-129 Symposium, Aflatoxin in Corn, Kansas City, Missouri
Mycotoxins: Cancer and Health, Pennington Biomedical Research Center, LSU
ISA Babcock & Incubadora Mexicana, Detoxification of Aflatoxins, Tehuacan, Mexico
International Groundnut Workshop, Detoxification of Aflatoxins, Hyderabad, India
World Congress on Foodborne Infections and Intoxications, Berlin, Germany.
IUPAC International Meeting on Mycotoxins and Phycotoxins, Mexico City.
Du Pont/ConAgra, Selective Chemisorption of Mycotoxins, Wilmington, Delaware.
International Meeting on Aviculture, Mexico City, Mexico.
Hoffmann-LaRoche Seminars: Rio, Port Allegre, Chapeko, Brazil
NutriBasics Seminars: Tapei, Taiwan; Penang, Maylasia; and Manilla, Philippines.
Hoffmann-LaRoche Seminars: Costa Rica
Eleventh International Symposium of the WAVFH, Bangkok, Thailand
National Congress of Agriculture, Aflatoxin Detoxification, Lima and Trujillo, Peru
International Mycotoxin Symposium, Curitiba, Brazil
Roche Seminars: Mexico City, Gadalajara, Monterrey, Mexico
TAMU Extension Aflatoxin Conference, Corpus Christi, Texas
Society of Toxicology (GCC), San Antonio, Texas
American Society of Agronomy, St. Louis, Missouri
American Feed Industry Insurance Co., St. Louis, Missouri
Associated Milk Producers, Inc., Dallas, Texas
Texas Association of Milk, Food, and Environmental Sanitarians, Austin, Texas
UT Health Science Center, Pharmacology and Toxicology, Houston, Texas
UM Medical Center, Pharmacology and Toxicology, Jackson, Mississippi
XX World's Congress on Poultry, Delhi, India
DuCoa Seminars: Taipai, Taiwan; Bangkok, Thailand; and Manila, the Philippines
USFGA Seminar: Santiago, Chile
NIEHS Superfund Basic Research Program - EPA Meeting, New York, NY
Gordon Conference on Mycotoxins and Phycotoxins, Plymouth, NH
USFGA Seminar: Dominican Republic
PHILSAN Seminar, Manila, the Philippines
Vicam Seminar, Boston, MA

CONSULTATION:

Kaiser Aluminum and Chemical Corp., Center for Technology, Pleasanton, CA.
Water's Analytical Associates, Milford, MA
Computer Sciences Corp., National Space Technology Laboratories, NSTL, MS.
Harshaw Filtrol Chemical Corporation, Cleveland, OH.
Neogen Diagnostics Corporation, East Lansing, MI.
Rialdon Diagnostics Corporation, Bryan, TX.
Papillon Agricultural Products, Inc., Easton, MD.
TerraTek Corporation, Salt Lake City, Utah
Engelhard Corporation, Cleveland, Ohio
Vicom, Watertown, Massachusetts
Ralston Purina International, St. Louis, Missouri
Ralston Purina International, Mexico City, Mexico
NutriBasics and Hoffmann-La Roche International
American Feed Industry Insurance Company
United States Feed Grain Association, Washington, D.C.

INTERNATIONAL PROGRAM ACTIVITIES:

Title XII/USAID Project - Mycotoxin Management

Country: Senegal, West Africa

Institut de Technologie Alimentaire (ITA) and Institut Senegalais De Recherches (ISRA) Agricoles, Dakar, Senegal, 1983-1995.

Objectives: Field-practical detection and detoxification of aflatoxin contaminated peanuts and peanut products.

Numerous scientific presentations and collaborations ongoing in other countries

INTERNATIONAL VISITING SCIENTISTS:

1994: Professor Norberto Lisker (Head of Seed Pathology); Ministry of Agriculture (ARO), The Volcani Center, Israel (Sabbatical).

1993: M.A. Abdel-Wahhab (Egyptian Scientific Channel System), National Research Center, Dokki, Cairo, Egypt (PhD candidate).

1991: Dr. Amadou Kane, Institut de Technologie Alimentaire, Dakar, Senegal, West Africa (USAID trainee).

1985: Dr. Amangone N'Doye, Institut Senegalais de Reserches Agricoles, Dakar, Senegal, West Africa (USAID trainee)

000427

FORMER GRADUATE STUDENTS:

[Redacted] (1982). [Redacted] Title: [Redacted]

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Technology for anti-toxins.

POSTDOCTORAL RESEARCH ASSOCIATES:

[REDACTED] : [REDACTED]

UNDERGRADUATE STUDENT WORKERS:

[REDACTED]
[REDACTED]

List A-6

000431

**Curriculum Vitae
Robert Henry Proctor**

Work Address

Home Address

Date and Place of Birth:

Education:

1980 - 1984 University of Victoria (British Columbia), B.Sc. (Honours)
in Biology

1984 - 1988 University of Wisconsin-Madison, M.S. in Plant Pathology

1988 - 1990 University of Wisconsin-Madison, Ph.D. in Plant Pathology

Research Experience:

1984 - 1990 Research Assistant/Graduate Student, Department of Plant
Pathology, University of Wisconsin-Madison

1990 - 1993 Research Associate, Mycotoxin Research Unit, USDA
Agricultural Research Service, National Center for Agricultural
Utilization Research (NCAUR)

1993 - 1996 Research Associate, Bioactive Constituents Research Unit
USDA/Agricultural Research Service, NCAUR

1996-Present Research Microbiologist; USDA/Agriculture Research, NCAUR

Professional Societies:

American Phytopathological Society

Canadian Phytopathological Society

International Society for Molecular Plant-Microbe Interactions

000432

Professional Advisory and Consulting Activities:

Reviewer for competitive grant for the following organizations:

USDA National Research Initiative Competitive Grants Program, 1996-1997.

The Israel Science Foundation, 1997.

Austrian Science Fund, 1997.

USDA, ARS Preharvest Control of Aflatoxins, 1997-2000

Ad-hoc reviewer for the following scientific journals: Canadian Journal of Botany, Canadian Journal of Plant Pathology, Current Genetics, Fungal Genetics and Biology, Mycopathologia, Preventive Medicine, and Phytopathology.

Speaking Invitations:

“Regulation of a Cytochrome P450 Monooxygenase Gene Involved in Biosynthesis of a Fungal Toxin” Midwest Cytochrome P450 Symposium, Purdue University, West Lafayette, IN, 1994.

“Trichothecene Toxins and Virulence of the Head Scab Fungus *Fusarium graminearum*” Department of Plant Pathology, University of Wisconsin, Madison, WI, 1995.

“Trichothecenes and Wheat Head Scab” NCR-184, Management of Head Scab of Small Grains meeting, St. Paul, MN, 1995.

“Mapping of Fumonisin Biosynthetic Genes in *Gibberella fujikuroi*,” Screening Methodology for Problematic Diseases, Annual Meeting of the American Phytopathological Society, Pittsburg, PA, 1995.

“Wheat Head Scab and Vomitoxin,” Conventional Breeding, Biological and Biotechnological Methods of Control, Grain Quality Workshop in New Orleans, LA, 1997.

“Genetics of Fumonisin Biosynthesis in *Gibberella fujikuroi* mating population A,” 20th Fungal Genetics Conference, Pacific Grove, CA, 1999.

“Genetics of Fumonisin Production,” Gordon Research Conference on Mycotoxins and Phycotoxins, Plymouth State College, Plymouth, NH, 1999.

“Genetics of Mycotoxin Biosynthesis in *Fusarium*,” International Symposium of Mycotoxicology, Chiba City, Japan, 1999.

“Genetic Analysis of the Role of Trichothecenes and Fumonisins in the Pathogenicity of *Fusarium*,” European Community COST Workshop on Mycotoxins in Plant Disease, Rome, Italy, 1999.

Publications

- Proctor, R. H. 1990 Phytoalexins and Dutch elm disease resistance. Ph.D. Thesis, University of Wisconsin-Madison. 132 pp.
- Hohn, T. M., Proctor, R. H. and Desjardins, A. E. 1992. Biosynthesis of sesquiterpenoid toxins by fungal pathogens. In Stahl, U. and Tudzynski, P., eds., *Molecular Biology of Filamentous Fungi*, VCH Publishers, New York. 276pp.
- Smalley, E. B., Raffa, K. F., Proctor, R. H. and Klepzig, K. D. 1993. Tree responses to infection by species of *Ophiostoma* and *Ceratocystis*. In Wingfield, M. J., Seifert, K. A., and Webber, J. F., eds., *Ceratocystis and Ophiostoma: Taxonomy, Ecology and Pathogenicity*, APS Press, St. Paul. 293 pp.
- Proctor, R. H. and Hohn, T. M. 1993. Aristolochene synthase: isolation, characterization, and bacterial expression of a sesquiterpenoid biosynthetic gene (*Ari1*) from *Penicillium roqueforti*. *J. Biol. Chem.* 268:4543-4548.
- Cane, D. E., Wu, Z., Proctor, R. H. and Hohn, T. M. 1993. Overexpression in *Escherichia coli* of soluble aristolochene synthase from *Penicillium roqueforti*. *Arch. Biochem. Biophys.* 304:415-419.
- Proctor, R. H., Guries, R. P. and Smalley, E. B. 1994. Lack of association between tolerance to the elm phytoalexin mansonone E and virulence in *Ophiostoma novo-ulmi*. *Can. J. Bot.* 72:1355-1364.
- Proctor, R. H., Hohn, T. M. and McCormick, S. P. 1995. Reduced virulence of *Gibberella zeae* caused by disruption of a trichothecene toxin biosynthetic gene. *Mol. Plant-Microbe Interact.* 8:593-601.
- Proctor, R. H., Hohn, T. M., McCormick, S. P. and Desjardins, A. E. 1995. *Tri6* encodes an unusual zinc finger protein involved in the regulation of trichothecene biosynthesis in *Fusarium sporotrichioides*. *Appl. Environ. Microbiol.* 61:1923-1930.
- Hohn T. M, Desjardins, A. E., McCormick, S. P. and Proctor, R. H. 1995. Biosynthesis of trichothecenes, genetic and molecular aspects, pp. 239-248. In Eklund, M., Richard, J. L., and Mise, K., eds., *Molecular Approaches to Food Safety*, Alaken, Inc., Ft. Collins, CO.
- Desjardins, A. E., Plattner, R. D., Proctor, R. H., Hohn, T. M. and McCormick, S. P. Genetic approaches to the role of fumonisin and trichothecene toxins of *Fusarium* in plant pathogens, pp. 311-318. In Eklund, M., Richard, J. L., and Mise, K., eds., *Molecular Approaches to Food Safety*, Alaken, Inc., Ft. Collins, CO.
- Desjardins A. E., Plattner, R.D. and Proctor, R. H. 1996. Genetic and biochemical aspects of fumonisin production. In Jackson, L. S., DeVries, J. W. and Bullerman, L. B., eds., *Fumonisin in Food*, Plenum Press, New York. 399pp.

000434

- Desjardins, A. E., Plattner, R. D. and Proctor, R. H. 1996. Linkage among genes responsible for fumonisin biosynthesis in *Gibberella fujikuroi* mating population A. *Appl. Environ. Microbiol.* 62:2571-2576.
- Desjardins, A. E., Proctor, R. H., Bai, G., McCormick, S. P., Shaner, G., Buechley, G. and Hohn, T. H. 1996. Reduced virulence of trichothecene-nonproducing mutants of *Gibberella zeae* in wheat field tests. *Molec. Plant-Microbe Interact.* 9:775-781.
- Alexander, N. J., Proctor, R. H., McCormick, S. P. and Plattner, R. D. 1997. Genetic and molecular aspects of the biosynthesis of trichothecenes by *Fusarium*. *Cereal Res. Commun.* 25:315-320.
- Proctor, R.H., Hohn, T. M. and McCormick, S. P. 1997. Restoration of wild-type virulence to *Tri5* disruption mutants of *Gibberella zeae* via gene reversion and mutant complementation. *Microbiology* 143:2583-2591.
- Hohn, T.M., Krishna, R. and Proctor, R. H. 1999. Characterization of a transcriptional activator controlling trichothecene toxin biosynthesis. *Fungal Genet. Biol.* 26:224-235.
- Proctor, R. H., Desjardins, A. E., Plattner, R. D. and Hohn, T. M. 1999. A polyketide synthase gene required for biosynthesis of fumonisin mycotoxins in *Gibberella fujikuroi* mating population A. *Fungal Genet. Biol.* 27:100-112.
- Proctor, R. H. 2000. *Fusarium* toxins: trichothecenes and fumonisins. In Cary, J. W., Linz, J. I., Stein, M. A. and Bhatnagar, D., eds., *Microbial Foodborne Diseases: Mechanisms of Pathogenicity and Toxin Synthesis*, Technomic Publishing Co. Inc., Lancaster, PA, in press.
- Desjardins, A. E. and Proctor, R. H. 2000. Biochemistry and genetics of *Fusarium* toxins. In Summerell, B., Leslie, J. F., Backhouse, D., Bryden, W. L. and Burgess, L. W., eds., *Fusarium: Paul E. Nelson Symposium*, APS Press, St. Paul, MN, in press.
- Proctor, R. H., Desjardins, A. E., Plattner, R. D. and Seo, J. A. 2000. Biosynthesis of fumonisins in *Gibberella fujikuroi* mating population A. *Mycotoxins* in press.
- Harris, L. J., Desjardins, A. E., Plattner, R. D., Nicholson, P., Butler, G., Young, J.C., Weston, G., Proctor, R. H. and Hohn, T. M. 1999. Possible role of trichothecene mycotoxins in virulence of *Fusarium graminearum* on maize. *Plant Dis.* 83:954-960

Abstracts

- Proctor, R. H. and Smalley, E. B. 1990. Effects of mansonone E on the growth of *Ophiostoma ulmi* and other fungi. Mycological Society of America Newsletter 41:33.
- Proctor, R. H. and Smalley, E. B. 1990. Lack of association between mansonone tolerance and virulence in *Ophiostoma ulmi*. Phytopathology 80:968.
- Proctor, R. H. and Hohn, T. M. 1991. Molecular cloning of a PCR fragment containing a portion of the aristolochene synthase gene from *Penicillium roqueforti*. Phytopathology 81:1162.
- Proctor, R. H. and Hohn, T. M. 1992. Characterization and regulation of the aristolochene synthase gene from *Penicillium roqueforti*. 6th International Symposium on Molecular Plant-Microbe Interactions.
- Proctor, R. H., Hohn, T. M. and McCormick, S. P. 1993. Analysis of the role of trichothecene toxins in the pathogenesis of *Gibberella zeae*. Fungal Genetics Newsletter 40A.
- Proctor, R. H., Hohn, T. M. and McCormick, S. P. 1993. Effect of *Tox5* gene disruption on the virulence of *Gibberella zeae*. Phytopathology 83:1416-1417.
- Proctor, R. H., Hohn, T. M., McCormick, S. P. and Desjardins, A. E. 1994. The *Fusarium sporotrichioides Tri6* gene encodes a zinc finger protein involved in the regulation of trichothecene biosynthesis. 5th International Mycological Congress, Vancouver, British Columbia.
- Proctor, R. H., Hohn, T. M., McCormick, S. P. and Desjardins, A. E. 1995. Reversion of a disrupted toxin biosynthetic gene restores high virulence in *Gibberella zeae*. Fungal Genetics Newsletter 42A:35.
- Proctor, R. H., Desjardins, A. E., Plattner, R. D., Hohn, T. M., Leslie, J. F., and Xu, J.-R. 1995. Mapping of fumonisin biosynthetic genes in *Gibberella fujikuroi*. Proceedings of the Annual Meeting of the American Phytopathological Society, Pittsburgh, PA.
- Desjardins, A. E., Proctor, R. H., McCormick, S. P. and Hohn T. M. 1995. Reduced virulence of a trichothecene nonproducing mutant of *Gibberella zeae* on field grown wheat. Fungal Genetics Newsletter 42A:35.
- Proctor, R. H. 1995. RAPD-Bulked segregant analysis based mapping of a *Gibberella fujikuroi* gene involved in fumonisin biosynthesis. Fungal Genetics Newsletter 42A:38.
- Proctor, R. H., Desjardins, A. E., McCormick, S. P. and Hohn, T. M. 1996. Trichothecene toxin production and virulence of *Fusarium graminearum* on wheat. 8th International Congress on Molecular Plant-Microbe Interactions, Knoxville, TN.

000436

- Proctor, R. H., Desjardins, A. E. and Plattner R. D. 1997. Analysis of a *Gibberella fujikuroi* mutant deficient in a hydroxylation step of fumonisin biosynthesis. Proceedings of the 19th Fungal Genetics Conference, Pacific Grove, CA.
- Proctor, R. H., Desjardins, A. E., Plattner R. D. and Hohn, T. M. 1998. Isolation of a polyketide synthase gene required for fumonisin biosynthesis in *Gibberella fujikuroi* mating population A. Gordon Research Conference, Molecular and Cellular Mycology.
- Proctor, R. H., Desjardins, A. E., Plattner, R. D. and Hohn, T. M. 1999. Genetics of fumonisin biosynthesis in *Gibberella fujikuroi* mating population A. 20th Fungal Genetics Conference, Pacific Grove, CA.
- Proctor, R. H., Desjardins, A. E., Plattner, R. D. and Hohn, T. M. 1999. A polyketide synthase gene required for the biosynthesis of fumonisin mycotoxins in *Gibberella fujikuroi* mating population A. 20th Fungal Genetics Conference, Pacific Grove, CA.
- Proctor, R. H., Desjardins, A. E. and Plattner, R. D. 1999. Genetics of fumonisin production. Gordon Research Conference, Mycotoxins and Phycotoxins.
- Proctor, R. H., Desjardins, A. E. and Plattner, R. D. 1999. Polyketide mycotoxin biosynthesis in *Fusarium*. Proceedings of the 1999 Society for Industrial Microbiology Annual Meeting.
- Proctor, R. H., Desjardins, A. E. and Plattner, R. D. 1999. Genetics of mycotoxin biosynthesis in *Fusarium*. International Symposium of Mycotoxicology, Chiba City, Japan.

000438

Section 2



2. INTRODUCTION

Novozym® 899 is a Novo Nordisk A/S trade name used for a xylanase enzyme preparation produced by submerged fermentation of a selected strain of *Fusarium venenatum* expressing the gene encoding a xylanase from *Thermomyces lanuginosus*.

The xylanase enzyme preparation is to be used in the food industry as a processing aid for baking applications. Xylanases, which are a types of pentosanases, are the most functional enzymes in the hemicellulase enzyme preparations, which are well known, in the baking industry (App. A, refs. 1-4).

The enzyme is an endo xylanase that hydrolyzes xylosidic linkages in an arabinoxylan backbone resulting in depolymerization of the arabinoxylan into smaller oligosaccharides. This increases the elasticity of the gluten network, improving handling and stability of the dough.

The information contained in this document presents the basis for Novo Nordisk's determination of the general recognition of safety of a xylanase enzyme preparation produced by a selected strain of *F. venenatum* expressing a xylanase gene from *T. lanuginosus*. This determination is corroborated and supported by an independent expert panel whose report is contained in Section 1. Sections 3-8 provide information on the enzyme preparation, the production microorganism, the manufacturing process, product specifications, and the intended use in baking applications, as well as an overall safety evaluation.

The overall safety evaluation (Section 8) includes a review of published and unpublished information supporting the safety of the production strain, the enzyme, and the manufacturing process as well as an evaluation of potential dietary exposure to the enzyme preparation and a summary and presentation of the toxicology studies done to confirm the safety of the product for its intended use. This evaluation concludes that this xylanase enzyme preparation is safe.

As indicated by Pariza and Foster (App. A, ref. 5), the safety of the production organism must be the prime consideration in assessing the probable degree of safety of an enzyme preparation intended for use in food. The production microorganism for Novozym 899, a selected strain of *F. venenatum* expressing a xylanase from *T. lanuginosus*, is described in Section 3. An essential aspect of the safety evaluation of food components derived from genetically modified organisms is the identification and characterization of the inserted genetic material (App. A, ref. 6-11). The genetic modifications used to develop the production microorganism are well-defined and are also described in Section 3. The safety of this production microorganism is considered in Section 8 and it is concluded that it is safe.

Section 4 includes a description of the xylanase activity and the data showing this xylanase to be equivalent to the previously determined

000441



3. PRODUCTION MICROORGANISM

3.1 Production Strain

The microbial production strain for Novozym 899, designated LyMC4.B, is a derivative of a wild type isolate of *F. venenatum* Nirenberg sp. nov. (App. B, ref. 1). The classification, described in Section 3.2, is based on morphological, physiological, and molecular taxonomic characteristics.

The genetically modified *F. venenatum* strain LyMC4.B is non-pathogenic non-toxicogenic, and meets the criteria for a safe production microorganism as described by Pariza and Foster (App. A, ref. 5) and several expert groups (App. A, ref. 6-11). These criteria include the identification and characterization of the host strain, plasmid vectors, and inserted genetic sequences, all described below. In addition, *F. venenatum* strain LyMC4.B complies with the OECD (Organization for Economic Co-operation and Development) criteria for GILSP (Good Industrial Large Scale Practice) microorganisms (App. A, ref. 14).

The *F. venenatum* production strain, LyMC4.B, was constructed by common transformation procedures using well-known plasmid vectors and well-characterized DNA sequences. Two plasmids were used in the strain construction, one a xylanase expression plasmid and the other a plasmid designed to incorporate a deleted replacement of the trichodiene synthase gene (*tri5*). The development of the production strain was evaluated at every step to assess incorporation of the desired functional genetic information and to ensure no unintended sequences were incorporated.

3.2 Recipient Microorganism

Accurate identification of *Fusarium* species has been problematic and has often resulted in conflicting and evolving species designations. Because many *Fusaria* are noted for the ability to produce various mycotoxins, precise species characterization and classification is necessary for any isolate to be used as a host for expression of enzymes for use in food processing and other industrial applications.

The recipient microorganism, *F. venenatum* strain MLY3, used in construction of the xylanase production strain is a derivative of the *F. venenatum* strain CC1-3 (App. B, ref. 2), a morphological mutant of the wild type *F. venenatum* strain A3/5. The taxonomic characteristics are:

Class:	Hyphomycetes
Order:	Hyphomycetales
Genus:	<i>Fusarium</i>
Section:	<i>Fusarium</i> (previously <i>Discolor</i>)
Species:	<i>venenatum</i>

The wild type strain is identified in the American Type Culture Collection (ATCC) as *F. graminearum* Schwabe ATCC 20334. This strain, originally isolated from soil in the United Kingdom (UK), is also referred to in the

000442

literature as strain A3/5 and has been deposited as *F. graminearum* in a number of international culture depositories including, the Imperial Mycological Institute, Surrey, UK (Accession No. 145245) (App. B, ref. 3).

This *Fusarium* strain, ATCC 20334 (UK Accession No. 145425), was approved by the UK Ministry of Agriculture, Fisheries, and Food as 'Quorn' mycoprotein (App. B, ref. 4) in 1985 and has since been sold in the UK under the registered trade name Quorn (App. B, ref. 5).

Dr. H. Nirenberg, a recognized international expert authority on the identification of different species in the genus *Fusarium*, has positively identified ATCC 20334 as *F. venenatum* (App. B, ref. 6), a conclusion supported by Dr. U. Thrane at the Danish Technical University (App. B, ref. 7) and further verified by the published work of Yoder and Christianson (App. B, ref. 3), and O'Donnell et al (App. B, ref. 8).

The *F. venenatum* strain CC1-3 is a morphological mutant that arose spontaneously during a Quorn fermentation of strain A3/5 (App. B, ref. 2). It has a highly branched morphology, forming dense colonies with slower colony radial growth rates than parental colonies.

F. venenatum strain MLY3 is a spontaneous mutant derivative of *F. venenatum* strain CC1-3. *F. venenatum* strain MLY3 supports higher expression levels of heterologous proteins than strain CC1-3.

3.3 Introduced DNA Sequences

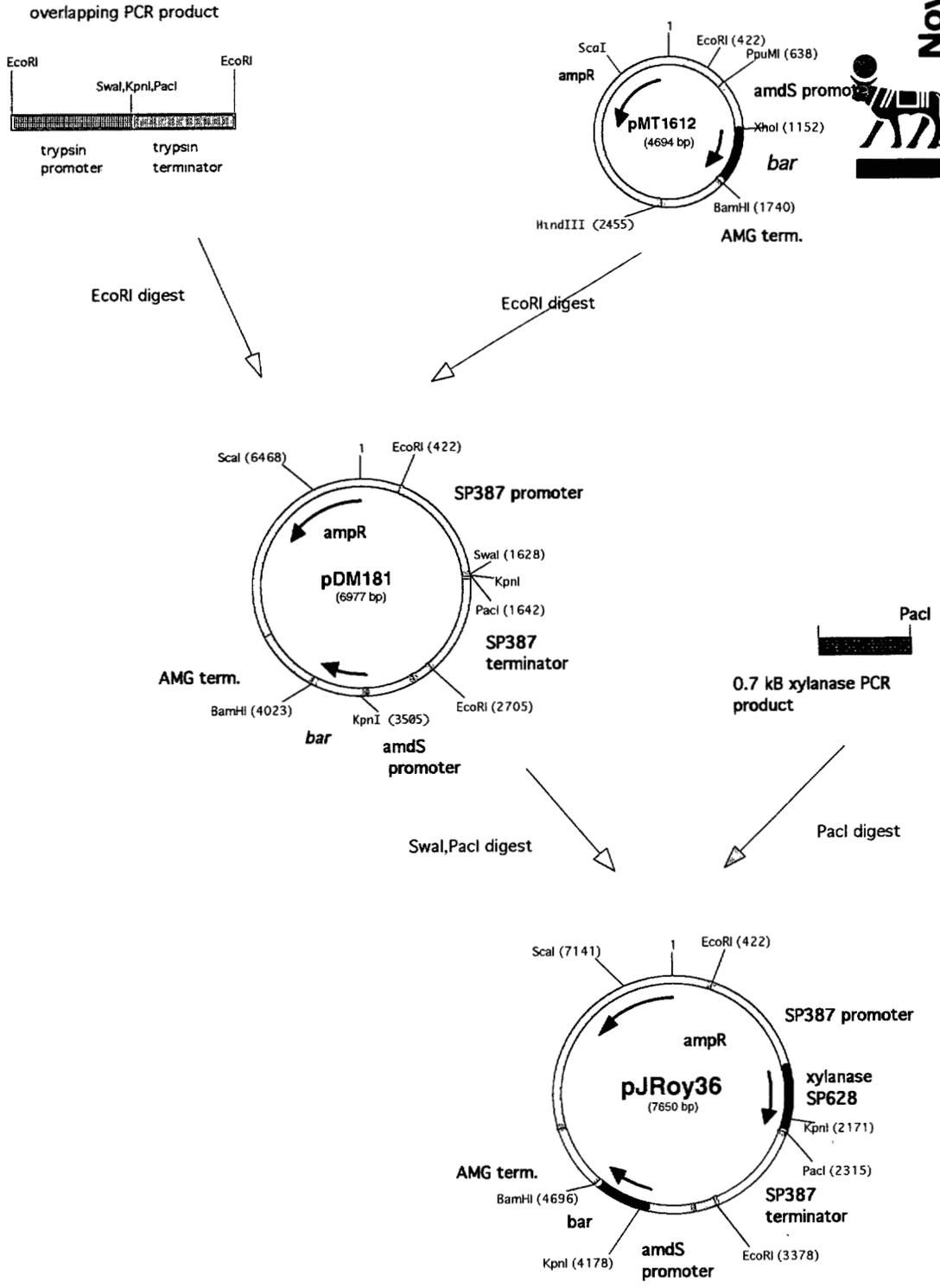
3.3.1 Xylanase expression plasmid pJRoy36 (see Figure 1)

Traditional molecular genetic and rDNA techniques were used to construct the 7.7 kb plasmid pJRoy36 used to obtain expression of *T. lanuginosus* xylanase in *F. venenatum*. Plasmid pJRoy36 has the following gene elements:

- 1.2 kb containing the *F. oxysporum* trypsin gene promoter
- 0.7 kb containing the xylanase gene from *T. lanuginosus*
- 1.1 kb containing the *F. oxysporum* trypsin gene terminator
- 4.7 kb containing *E. coli* vector pUC 19 and a 1.8 kB fragment with the *A. nidulans amdS* promoter, the *S. hygroscopicus (bar)* gene and the *A. niger* AMG terminator.



Figure 1: Construction of xylanase expression vector pJRoy36





3.3.1.1 origin of the *F. oxysporum* trypsin promoter and terminator

The trypsin promoter and terminator sequences are from *F. oxysporum* Schlechtendahl:Fries, DSM 2672 (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). *F. oxysporum* has been described by Nelson et al. (App. B, ref. 9).

The technique of spliced overlap extension was used to fuse 1.2 kb of the *F. oxysporum* trypsin promoter to 1.1 kb of the *F. oxysporum* trypsin terminator. A polylinker containing *Swa*I, *Kpn*I and *Pac*I restriction sites was inserted between the promoter and terminator as part of the overlapping strategy. At the 5' end of the promoter a *Xho*I site was added and the native *Eco*RI site was preserved. At the 3' end of the terminator *Eco*RI, *Hind*III and *Nsi*I sites were incorporated.

Two PCR (polymerase chain reaction) fragments were generated from the trypsin promoter and terminator sequences in plasmid pJRoy20 (App. B, ref. 10). One PCR fragment contained from base pair position -1208 to position -1 of the *F. oxysporum* trypsin promoter plus the 25 base pair polylinker containing the *Swa*I, *Kpn*I and *Pac*I restriction sites. The other PCR fragment contained from base pair position -5 to position -1 of the *F. oxysporum* trypsin promoter, the same 25 base pair polylinker, and 1060 base pairs of the 3' untranslated region of the *F. oxysporum* trypsin gene (terminator region).

The final 2.3 kb overlapping PCR fragment which contains from base pair position -1208 to position -1 of the *F. oxysporum* trypsin promoter, the 25 base pair polylinker, and 1060 base pairs of the *F. oxysporum* trypsin terminator was made using the first PCR (promoter) reaction and the second (terminator) reaction as templates.

3.3.1.2 origin of the *bar* selectable marker

The *Streptomyces hygroscopicus* phosphinothricin acetyltransferase (*bar*) gene (App. B, ref. 11) was used as a selectable marker for the xylanase expression plasmid pJRoy36. *F. venenatum* strains expressing the *bar* gene can grow on a medium containing phosphinothricin, the active ingredient in the herbicide BASTA® (Hoechst). Safety assessments have been done on the phosphinothricin acetyltransferase protein and the *bar* gene DNA in association with their use in the construction of transgenic plants resistant to the phosphinothricin herbicide (see Section 8.3).

3.3.1.3 origin of other expression vector sequences

Plasmid pMT1612 (see Figure 1) is composed of the *bar* gene with associated regulatory sequences from the *Aspergillus nidulans* *amdS* promoter (App. B, ref. 12) and the *A. niger* AMG terminator (App. B, ref. 13) inserted into the *Escherichia coli* plasmid vector pUC19 (App. B, ref. 14).



3.3.1.4 construction of the intermediate plasmid pDM181

The intermediate plasmid pDM181 was created by ligating the *EcoRI* digested spliced trypsin promoter/terminator fragment into the *EcoRI* digested plasmid pMT1612. Plasmid pDM181 contains the spliced trypsin promoter/terminator, the *bar* selectable marker gene, and pUC19 vector DNA sequences. The trypsin promoter and terminator sequences are designated SP387 promoter and terminator on the pDM181 plasmid map in Figure 1.

3.3.1.5 origin of the xylanase gene

The origin of the xylanase gene is *T. lanuginosus* strain GH107 (synonym *Humicola lanuginosa*) (App. B, refs. 15, 16). *T. lanuginosus* is an ubiquitous, thermophilic fungi.

The 675 bp *T. lanuginosus* xylanase coding sequence incorporated into the pJRoy36 expression plasmid was generated by PCR, using a *T. lanuginosus* xylanase cDNA sequence as a template. PCR primers were used to introduce the sequence CCACC at the 5' end and a *PacI* site at the 3' end of the xylanase coding sequence.

This xylanase coding sequence was also used in the construction of the production microorganism for a xylanase preparation produced by *A. oryzae* expressing the xylanase from *T. lanuginosus* (Novo Nordisk trade name, Pentopan Mono™) that was the subject of a 1997 meeting between FDA and Novo Nordisk (see Section 4.1, Section 8.2, and App. C, ref. 1).

3.3.1.6 construction of the expression plasmid pJRoy36

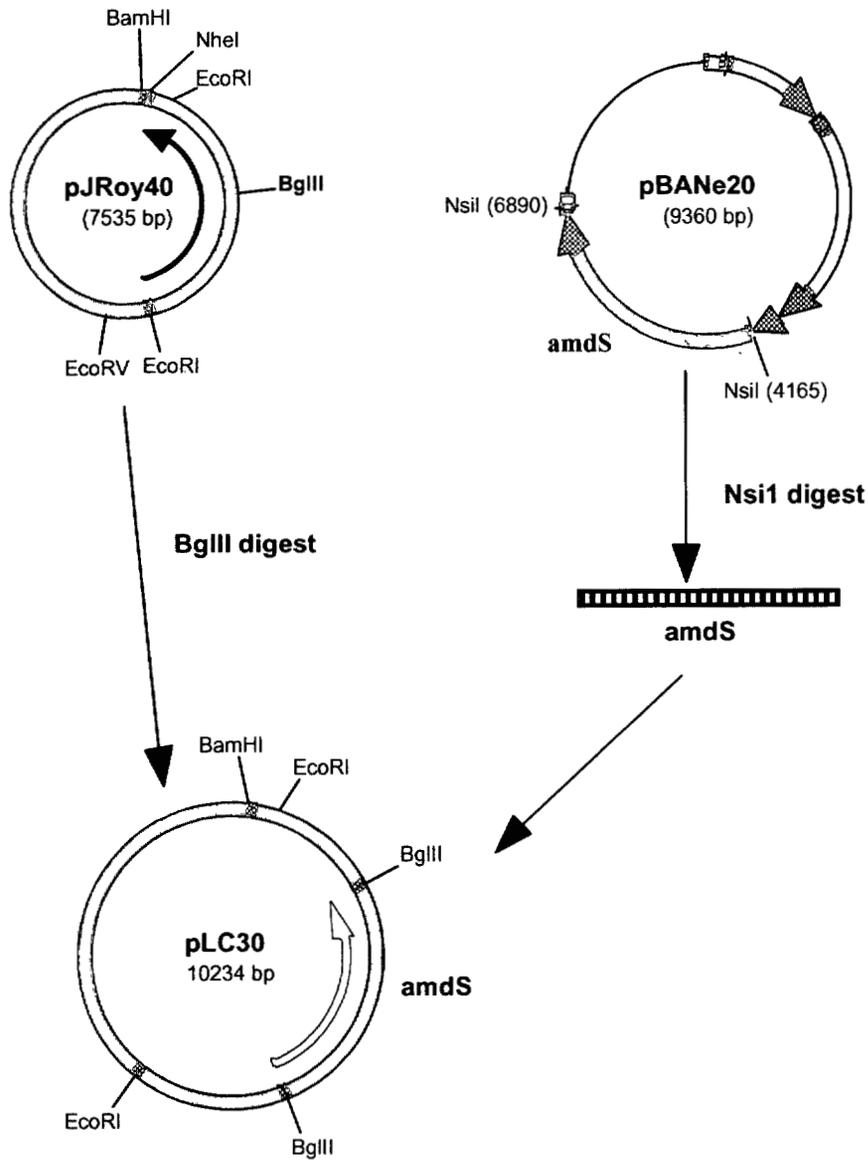
The 0.7 kb xylanase coding sequence fragment was digested with *PacI* and ligated to *SwaI/PacI* digested intermediate plasmid pDM181 yielding the xylanase expression plasmid pJRoy36 in which the xylanase gene is under transcriptional control of the trypsin promoter and trypsin terminator from *F. oxysporum*. The trypsin promoter and terminator sequences are designated SP387 promoter and terminator on the pJRoy36 plasmid map in Figure 1.

3.3.2 *tri5* replacement vector pLC30 (Figure 2)

The 10.2 kb *tri5* replacement vector, pLC30, is similar to plasmid PLC31b described in a recently published technique for deleting the *F. venenatum tri5* gene (App. B, ref. 17). Plasmid pLC30 contains the following genetic elements:

- 1.7 kb from the 5' region of the *F. venenatum tri5* gene
- 2.7 kb fragment containing the *amdS* gene of *A. nidulans*
- 1.5 kb from the 3' region of the *F. venenatum tri5* gene
- 4.3 kb cloning vector plasmid pZL1

Figure 2: Construction of the *tri5* deletion replacement vector pLC30





The *amdS* coding region of *A. nidulans* (App. B, ref. 12) was cloned into the *Bgl*III site of pJRoy 40 (App. B, ref. 17) to create pLC30 (see Figure 2) in which the 2.7 kb *A. nidulans amdS* sequence from pBANE20 is placed between the 5' and 3' regions of the *tri5* gene of *F. venenatum* and substitutes for the *tri5* gene coding sequence.

A. nidulans amdS (acetamidase) is a commonly used selectable marker gene used in fungal cloning (App. B, ref. 12). The *A. nidulans* acetamidase enzyme enables *A. nidulans* (and other fungi) to use acetamide and other amides as sole nitrogen and carbon sources. Acetamide, CH_3CONH_2 , is hydrolyzed by acetamidase to $\text{CH}_3\text{COOH} + \text{NH}_3$. These compounds, acetic acid and ammonia, can be used as a carbon and nitrogen source for the growth of the organism.

3.4 Construction of the Production Strain LyMC4.B

3.4.1 Generation of the xylanase transformant strain, JRoy36-19.B

Protoplasts of *F. venenatum* strain MLY3 were incubated with the xylanase expression plasmid, pJRoy36. Transformants were selected by growth on a medium containing phosphinothricin. Phosphinothricin resistant transformants were screened for xylanase activity. A single transformed colony (JRoy36-19.B) was selected.

3.4.2 Generation of the *tri5* deleted strain LyMC4.B

The *tri5* replacement plasmid vector, pLC30 (see Figure 2), was digested with *Eco*R1. The resulting 5.6 kb fragment containing the 5' and 3' regions of the *F. venenatum tri5* gene flanking the *A. nidulans amdS* gene was isolated. This purified fragment was used to transform the xylanase expressing strain JRoy36-19.B. Transformants were selected on minimal medium containing acetamide as the sole nitrogen source and individual isolates were assayed for 4,15 diacetyxyscirpenol (DAS) production and xylanase production. In addition, Southern analysis was performed on selected strains.

Strain LyMC4 was found to generate no detectable DAS, and to produce high levels of xylanase in shake flask and bench-top fermentation tests. A single spore isolate of strain LyMC4 was selected as the production strain and designated strain LyMC4.B.

3.4.3 Southern analysis of *tri5* deleted strains

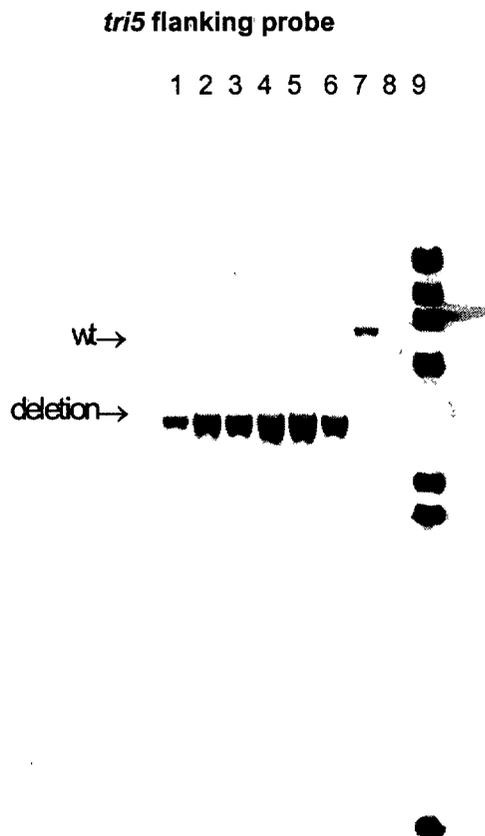
Southern hybridization was performed on the parent strain (JRoy36-19.B) as well as potential deletants and single spore isolates of deletants to test for deletion of the *tri5* coding sequence (Figures 4a and 4b). Total DNA was digested with *Sph*I and *Dra*I, electrophoresed on an agarose gel, and transferred to a nylon membrane. *Sph*I cuts within both the 5' and 3' region of the *F. venenatum tri5* gene and *Dra*I cuts within the 3' region of the *F. venenatum tri5* gene and within the *A. nidulans amdS* gene. Two probes were utilized to confirm the absence of the *tri5* coding region in LyMC4.B.

000448



A probe homologous to a portion of the 5' flanking DNA of the *tri5* coding sequence was hybridized to a Southern blot (Figure 4a). A shift in the size of the hybridizing band from 5.9 kb (corresponding to the wild type (wt) sequence) to 3.1 kb (corresponding to the *amdS* replaced sequence) indicated that the *tri5* gene had been replaced by the *amdS* gene. This shift was apparent in strain LyMC4.B (lane 5).

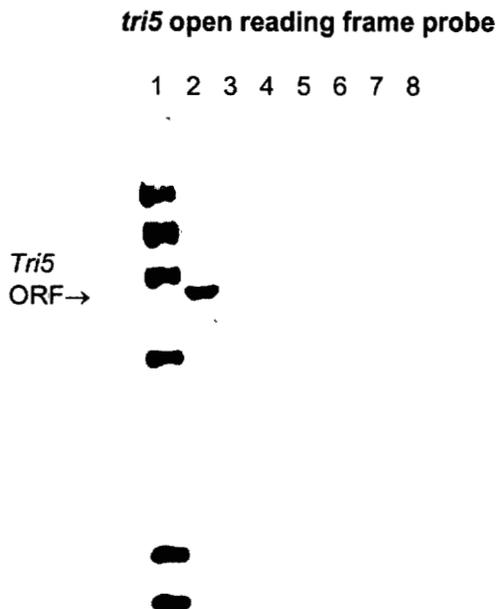
Figure 4a: Southern analysis of *tri5*-deleted strains.



***tri5* flanking probe.** Lane 1, LyMC19.5; lane 2, LyMC19.2; lane 3, LyMC19; lane 4, LyMC4.C; **lane 5, LyMC4.B**; lane 6, LyMC4; lane 7, JRoy36-19B; lane 8, empty; lane 9, fluorescein-labelled lambda-*Hind*III molecular weight marker.

In addition, a probe derived from within the *tri5* coding region was prepared, and was used to hybridize to a Southern blot (Figure 4b). A hybridizing band is present in the parent strain JRoy36-19.B (lane 2). The absence of a hybridizing band in LyMC4.B (lane 4) confirms that the *tri5* gene has been deleted in this strain.

Figure 4b: Southern analysis of *tri5*-deleted strains.



***tri5* open reading frame probe.** Lane 1, fluorescein-labelled lambda-*Hind*III molecular weight marker; lane 2, JRoy36-19B; lane 3, LyMC4; lane 4, LyMC4.B; lane 5 LyMC4.C; lane 6, LyMC19; lane 7, LyMC19.2; lane 8, LyMC19.5.

3.5 Antibiotic Resistance Gene

Prokaryotic and eukaryotic sites and signals for effective and functional gene expression and gene product synthesis are different. This basic principle of molecular cell biology and genetics is generally recognized within the scientific community (App. A, ref. 15). Plasmid pJRoy36 contains the β -lactamase gene, *bla*, encoding resistance to the antibiotic ampicillin. This gene is prokaryotic in origin and lacks the appropriate sites and signals (promoter, ribosome binding site, etc.) to be functionally expressed when integrated in an eukaryotic chromosome.

In addition, the prokaryotic *bla* gene lacks appropriate eukaryotic signal sequences and processing signals necessary for secretion and export. Therefore, any β -lactamase potentially produced by expression of the *bla* gene would be intracellularly localized and not be present in the final product, the xylanase enzyme preparation.

Novo Nordisk has confirmed the absence of *bla* gene expression in fungal systems by several tests of *A. oryzae* production strains containing the *bla* gene which have not shown any evidence of *bla* gene expression or β -lactamase synthesis.

000450



3.6 Stability and Transfer Capability of the Introduced DNA

The presence and configuration of the introduced DNA sequences in the production strain, *F. venenatum* LyMC4.B, were determined by Southern hybridization analysis to assess the stability and potential for transfer of genetic material. No instability of the introduced DNA sequences strain was observed. The transformed plasmid DNA is stably integrated into the *F. venenatum* chromosome and, as such, is mitotically stable and is poorly mobilizable for genetic transfer to other organisms (App. A, ref. 14).

In addition, a test batch produced by the production strain, *F. venenatum* LyMC4.B, was assayed for the presence of recombinant DNA sequences by dot blot hybridization assay and for the presence of recombinant DNA sequences capable of transformation. No recombinant DNA sequences were detected at a detection limit of 0.1 ng DNA / g. No recombinant DNA sequences capable of transformation were detected in a 10 mg sample.

3.7 Plant Pathogenicity

F. venenatum is a saprophytic fungus found in soil and is also known to occur on *Humulus lupulus*, *Solanum tuberosum*, *Spinacia oleracea*, *Triticum aestivum*, *Zea mays* (App. B, ref. 1). The *F. venenatum* production strain LyMC4.B is derived from the wild type *F. venenatum* strain A3/5 (=ATCC 20334) (see Section 3.2). *F. venenatum* strain ATCC 20334 has been tested for plant pathogenicity on potatoes, wheat, and barley and was found to be non-pathogenic on these plants.

3.8 Human Pathogenicity

F. venenatum is not considered a human pathogen (App. B, ref. 18).

The *F. venenatum* production strain LyMC4.B is derived from the wild type *F. venenatum* strain A3/5 (=ATCC 20334) (see Section 3.2). The *F. venenatum* strain ATCC 20334 has a reported history of safe industrial use. Biomass from this strain is marketed as Quorn Mycoprotein and has been a readily available human food source in England since 1985 (App. B, ref. 5). The Quorn Mycoprotein was concluded to be a safe food ingredient in an expert panel report, that has been used to support a Food Additive Petition in the US, FAP 6A3930 (App. B, ref. 19, pers. comm.).

3.9 Mycotoxins

The mycotoxins / secondary metabolites, that may potentially be produced by *F. venenatum*, are the trichothecenes, the culmorins, the enniatins and the fusarins. Laboratory trials under optimum conditions for specific mycotoxin production, involving isolates obtained from different culture collections of the original *F. venenatum* A3/5 (=ATCC 20334), the morphological mutant *F. venenatum* CC1-3, and positive (*F. venenatum*) as well as negative (*F. torulosum*) control strains, show that all the *F. venenatum* isolates are able to produce the trichothecene, diacetoxyscirpenol (DAS).



The ability to produce DAS and related compounds were effectively interrupted by means of gene deletion and based on the extensive investigations and literature surveys that are outlined below, it is concluded that the production strain *F. venenatum* LyMC4.B does not produce secondary metabolites of toxicological concern to humans or higher animals.

3.9.1 Trichothecenes

The gene encoding trichodiene synthase (*tri5*) was deleted in LyMC4.B, thereby rendering it incapable of producing secondary metabolites within the trichothecene pathway (App. B, ref. 17). This was confirmed in a study of the secondary metabolites produced by a number of strains of *F. venenatum* (App. B, ref. 20). Under optimum conditions for specific mycotoxin production, wild type *F. venenatum* isolates produced the trichothecene DAS and related metabolites isotrichodermin and isotricodermol, the modified trichothecenes sambucinol and apotrichothecene, the sesquiterpenes culmorin and culmorone, and derivatives and trace amounts of the cyclic peptide enniatin B. Strains without a functional trichodiene synthase gene produced no trichothecenes or modified trichothecenes.

3.9.2 Culmorins and Enniatins

Under optimum conditions for specific mycotoxin production, LyMC4.B is capable of producing minor amounts ($\mu\text{g/l}$ level) of metabolites of the culmorin family and trace amount of enniatin B (App. B, ref. 20). The levels were estimated to be reduced compared to wild type isolates.

Reports on the toxicity of culmorin and related compounds (App. B, ref. 21) and enniatins (App. B, ref. 22) indicate that these compounds have low toxicity to higher animals.

Based on the combination of the low levels that may potentially be expressed and the low toxicity profile, it is concluded that culmorins and enniatin do not present a toxicological concern for the xylanase enzyme preparation from the selected *F. venenatum* strain, LyMC4.B, expressing the *T. lanuginosus* xylanase gene.

3.9.3 Fusarins

It has been reported that *F. venenatum* can be grown under conditions capable of inducing production of fusarin C (personal communication). Laboratory experiments, with *F. venenatum* A3/5 (=ATCC 20334), *F. venenatum* LyMC4.B, and positive (*F. sambucinum*) as well as negative (*F. solani*) control strains, showed that while fusarin C may be produced in ($\mu\text{g/l}$ level) by *F. sambucinum* and *F. venenatum* A3/5 (=ATCC 20334) and LyMC4.B and under inducing conditions, there is no indication of any fusarin production under normal industrial enzyme fermentation conditions with the production strain *F. venenatum* LyMC4.B.

The fermentation conditions, used during enzyme production, are different from the conditions known to induce fusarin biosynthesis, as deduced from a literature survey on fusarin C (App. B, ref. 23) and personal communication. The major differences are vigorous aeration, a constant supply of carbon and nitrogen, and addition of vitamins and trace metals used for industrial fermentation for enzyme production. In particular, the addition of vitamins and trace metals is known to down-regulate fusarin synthesis in other *Fusaria* (App. B, ref. 23).

A literature survey of toxicity data on fusarin C concludes, that the acute toxicity to humans is low and that the carcinogenic potential is not clarified due to lack of adequate studies (App. B, ref. 24).

Because the extremely low levels of fusarin C are only attained under specific inducing conditions and there is no indication of any synthesis under normal industrial enzyme fermentation conditions, it is concluded that fusarin C does not present a toxicological concern for the xylanase enzyme preparation from the selected *F. venenatum* strain expressing the *T. lanuginosus* xylanase gene.



000454



4. ENZYME IDENTITY and CHARACTERIZATION

4.1 Enzyme Identity

The primary enzyme activity in Novozym 899 is xylanase. According to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUB), "Enzyme Nomenclature 1992", (App. C, ref. 2) the xylanase is classified as:

Generic name:	carbohydrase
IUB nomenclature:	endo-1,4- β -xylanase
IUB No.:	EC 3.2.1.8

Xylanase hydrolyses the beta-(1,4)-D-xylosidic linkages in the arabinoxylan backbone. Arabinoxylans are highly branched xylans that are found in various cereals, and they exist both in a soluble and an insoluble form. Novozym 899 has activity towards both forms.

Identical xylanase genetic coding sequences were used in the construction of the production strains for Novozym 899 and Pentopan Mono (see Section 3.3.1.5). Novozym 899 (App. C, ref. 3) is the *T. lanuginosus* xylanase expressed in a *F. venenatum* host strain and Pentopan Mono (App. C, ref. 4) is the identical *T. lanuginosus* xylanase expressed in an *A. oryzae* host strain. The Novozym 899 enzyme product is substantially equivalent to Pentopan Mono enzyme product.

Substantial equivalence between the xylanase enzyme component of Novozym 899 and Pentopan Mono is evidenced by use of the identical genetic protein coding sequence, which was confirmed by means of N-terminal amino acid sequencing and further documented by conventional characterization techniques of the enzymes.

When assayed by SDS-PAGE, a predominant enzyme protein band, corresponding to a molecular weight of 24 kDa, is found in both preparations. The protein banding pattern obtained by isoelectric focusing of Novozym 899 is included in that obtained from Pentopan Mono. Immunochemical identity of the enzyme components from Novozym 899 and Pentopan Mono was demonstrated by means of crossed immunoelectrophoresis, where both enzyme components were assayed using antibodies raised against Novozym 899 and antibodies raised against Pentopan Mono.

In addition, baking performance tests demonstrated that Novozym 899 and Pentopan Mono perform identically with respect to measured bread and dough parameters.

4.2 Enzyme Activity

The activity of Novozym 899 is standardized in xylanase units (FXU (Farbe Xylanase Unit)) as defined in the Novo Nordisk method EB-SM-036.02/01. The method is based on enzymatic hydrolysis of a high-molecular weight AZO-coupled wheat xylan followed by mixing with ethanol. The amount of blue-colored low molecular weight xylan fragments made soluble in ethanol as a result of the xylanase activity is measured spectrophotometrically and compared to an internal standard. The activity of the commercial product Novozym 899 is standardized at 2500 FXU/g.

000456



5. MANUFACTURING PROCESS

Novozym 899 is fermented, recovered and formulated according to well-established standard manufacturing processes in the enzyme industry (App. D, ref. 1-3). The following section describes the details used in the manufacturing of Novozym 899. Novozym 899 is manufactured in accordance with current good manufacturing practices (cGMP) and the quality management system used in the manufacturing process complies with ISO 9001.

5.1 Raw Materials

All raw materials used for fermentation and for recovery are either approved food additives or safe for use in food and conform to Food Chemicals Codex (FCC) (App. A, ref. 16) specifications except those raw materials which do not appear in the FCC. For those not appearing in the FCC, internal specifications have been made in line with FCC requirements. On arrival at Novo Nordisk, the raw materials are sampled by the Quality Control Department and subjected to the appropriate analyses to ensure their conformance to specifications.

5.2 Fermentation

Novozym 899 is produced by a contained system of submerged fed-batch pure culture fermentation of the genetically modified strain, LyMC4.B, of *F. venenatum*, carrying the *T. lanuginosus* xylanase gene (see Section 3).

5.2.1 Raw materials for fermentation

The production strain is grown in a medium consisting of compounds providing an adequate supply of carbon and nitrogen plus minerals and vitamins necessary for growth.

The choice of raw materials used in the fermentation process (inoculum, seed fermentor, main fermentor, dosing) of Novozym 899 are listed below.

Carbohydrates (e.g. glucose, sucrose, maltose, starch hydrolysates)

Vegetable protein (e.g. soybean meal, potato protein)

NaNO₃

Urea

Yeast extract

Citric acid

Salts (e.g. K₂SO₄, K₂HPO₄, KH₂PO₄, (NH₄)₂HPO₄, MgSO₄)

Trace metals

Vitamins

Alkali and acid for pH adjustment

phosphinothricin-ammonium

Antifoams (e.g. polyoxyethylene-polyoxypropylene copolymer)



5.2.2 Control of fermentation environment

All equipment is carefully designed and constructed to prevent contamination by foreign microorganisms.

All valves and connections not in use for the fermentation are sealed by steam at more than 120°C.

After sterilization a positive pressure of more than 0.2 atmosphere is maintained in the fermentor.

The air used for aeration is sterilized by passing a sterile filter.

The inside of each fermentor is carefully cleaned between fermentations by means of a high-pressure water jet and inspected after the cleaning procedures have been completed.

5.2.3 Stock culture

The stock culture used in the fermentation is lyophilized *F. venenatum*, as described in section 3.

Each new batch of stock culture is thoroughly controlled before use. The parameters controlled are the following:

- Identity of the microorganism
- Absence of foreign microorganisms
- Enzyme-generating ability

5.2.4 Preparation of inoculum used in the fermentation

The inoculum flask containing the prepared medium is autoclaved and controlled. Only approved flasks are used for inoculation.

The stock culture suspension is injected aseptically into the inoculum flask and mixed with the medium in the flask.

The herbicide phosphinothricin-ammonium (BASTA® (Hoechst)) is used only in the inoculation medium, in a concentration of 5 g/l, in order to maintain the selection pressure on the introduced DNA sequences. The safety evaluation for the use of this substance and the conclusion that it presents no safety concern are described in Section 8.3.

Growth is obtained in the inoculum flask and the following parameters are controlled:

The inoculum flask

Process operation	Control parameter	Action
Preparation of seed material	Strain id	The number of the used vial is registered on the inoculum flask and in the batch documentation
	Growth conditions	The inoculum flask is maintained at approx. 30°C for 4-7 days
	Microbiological analyses	A sample from the inoculum flask is controlled microscopically.
	Seed transfer criteria	Sufficient growth Approved microbiological analysis

When a sufficient amount of biomass is obtained and when the microbiological analyses are approved, the inoculum flask is used for inoculating the seed fermentor.

5.2.5 Seed fermentor

Raw materials for the medium are mixed with water in a mixing tank of stainless steel. The mixture is then heat sterilized in the fermentor. The seed fermentor is inoculated by transferring a suspension of cells from the inoculum flask through sterile tubing and a special connector.

Seed fermentor

Process operations	Control parameters	Action
Preparation of media	Weight adjustment	The amount of the raw materials used are adjusted & registered
	Sterilization	The media is sterilized at min. 120°C for min. 45 minutes
	Analysis after sterilization	RI %* and pH is measured. If necessary pH is adjusted
Fermentation in the seed fermentor	Temperature	The temperature is controlled to setpoint $\pm 2.0^{\circ}\text{C}$
	Agitation	Vigorous agitation is used
	Aeration	The media is aerated by sterile airflow
	Pressure	The pressure is kept above 0,2 atm at all time
	pH	Controlled to setpoint ± 1
	Dry substance	RI% determined at regular intervals
	Microbiological analysis	Samples are controlled just prior to inoculation and seed transfer
	Seed transfer criteria	Sufficient growth Approved microbiological analysis

* RI = Refractive Index



5.2.6 Main fermentor

Raw materials for the medium are mixed with water in a mixing tank. Sterilization is carried out in the main tank at min. 120°C for min. 45 minutes.

When vigorous growth has developed in the seed fermentor the culture is transferred aseptically to the main fermentor.

The fermentation in the main tank is run as a normal submerged fed-batch fermentation.

Main fermentor

Process operation	Control parameter	Action
Preparation of media	Weight adjustment	The amounts of the raw materials are adjusted and registered
	Sterilization	The media is sterilized at min. 120°C for min. 45 minutes
	Analysis after sterilization	pH and RI % is measured. If necessary pH is adjusted
Fermentation	Temperature	The temperature is controlled to setpoint $\pm 2.0^{\circ}\text{C}$
	Agitation	Vigorous agitation is used
	Aeration	The media is aerated with sterile airflow
	Pressure	The pressure is kept above 0,2 atm
	pH	Controlled to setpoint ± 1
	Feed start	pH > setpoint and increasing dissolved oxygen
	Feed rate	The rate is controlled and adjusted to avoid accumulation of carbohydrate
	Analysis	pH and RI % are measured at regular intervals
	Enzyme activity	The enzyme activity is measured at regular intervals
	Microbiological analysis	Samples are controlled just prior to inoculation and at regular intervals during fermentation

5.2.7 Microbial control

Samples are withdrawn from both the seed fermentor and the main fermentor:

- a) before inoculation
- b) at regular interval during cultivation
- c) before transfer/harvest



The methods for testing during all steps are the following:

- a) microscopy
- b) plating on a nutrient agar and incubated 24-48 hours.

Growth characteristics are observed macroscopically and microscopically.

The fermentation is declared "contaminated" if one of the following conditions are fulfilled:

- 1) Microscopical examination of at least two independent samples shows presence of foreign (contaminating or infecting) organisms.
- 2) Two samples taken within a six hour interval show presence of foreign (contaminating or infecting) organisms after growth on nutrient media.

Any contaminated fermentation is rejected.

5.3 Recovery

The recovery process is a multistep operation, designed to separate the desired enzyme from the microbial biomass and partially purify, concentrate, and stabilize the enzyme.

5.3.1 Raw materials for recovery

The choice of raw materials used in the recovery process of Novozym 899 are listed below:

Diatomaceous earth
Inorganic acids and bases for pH adjustment
Antifoams (e.g. polyoxyethylene-polyoxypropylene copolymer)
Sodium chloride

5.3.2 Primary separation

The cell mass and other solids are separated from the broth by well established techniques such as precoat vacuum drum filtration and centrifugation. The precoat used in the filter and the filter aid used in the process is diatomaceous earth (diatomite or perlite).

The primary separation is performed at a well defined pH and temperature range.



5.3.3 Concentration

A combination of ultrafiltration and evaporation is applied for concentration and further purification. Both ultrafiltration and evaporation are well established techniques. The ultrafiltration is applied to fractionate high molecular weight components from low molecular weight impurities and is used to increase the activity/dry matter ratio. Evaporation is used to increase the activity.

The pH and temperature are controlled during the concentration step, which is performed until the desired activity and activity/dry matter ratio has been obtained.

5.3.4 Pre- and germ filtration

For removal of residual production strain cells, and as a general precaution against microbial degradation, filtration on a dedicated germ filtration media is applied. Prefiltration is included when needed. The precoat of the filter and the filter aid used is diatomaceous earth.

The filtrations are performed at well defined pH and temperature intervals, and results in an enzyme concentrate solution free of the production strain and insoluble substrate components from the fermentation.

5.3.5 Preservation and stabilization

In order to prevent microbial degradation a stabilizer is introduced immediately after the germ filtration.

The quantity of the stabilizer is calculated on basis of the expected amount of concentrate after the final concentration.

The enzyme concentrate is stabilized by adding sodium chloride.

5.3.6 Final concentration

In case the concentration is too low to reach the target yield for the final product, a further concentration may be carried out by evaporation.

5.3.7 Granulation

The liquid concentrate is mixed with granulation aids (stabilizers/binders) such as dextrin and sorbitol syrup and spray dried by means of atomization into a fluidized spray dryer.

The powder from the primary drying zone is directed into an integrated fluid bed for agglomeration and further drying. The product is discharged continuously after sieving.

The product is standardized to the declared enzyme activity by addition of wheat flour.

000463



6. COMPOSITION and SPECIFICATIONS

6.1 Composition

Novozym 899 is presently available in the following formulation:

Enzyme solids (TOS [*])	approx.	4%
Ash (mainly NaCl)	approx.	4%
Water	approx.	10%
Dextrin	approx.	4%
Sorbitol	approx.	1%
Wheat solids	approx.	77%

*TOS = Total Organic Solids, defined as: 100% - water - ash - diluents

6.2 Specifications

Novozym 899 meets the general and additional requirements for enzyme preparations as outlined in the monograph on enzyme preparations in FCC (App. A, ref. 16). In addition, Novozym 899 meets the general specifications for enzyme preparations used in food processing outlined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (App. A, ref. 17).

The complete specifications established by Novo Nordisk are:

Xylanase activity, FXU/g	according to declaration
Heavy metals [*]	not more than 30 ppm
Lead [*]	not more than 5 ppm
Arsenic [*]	not more than 3 ppm
Total viable count/g	not more than 5×10^4
Total coliforms/g	not more than 30
Enteropathogenic <i>E. coli</i> /25 g	negative by test
<i>Salmonella</i> /25 g	negative by test
Antibiotic activity [*]	negative by test
Mycotoxins [*]	negative by test
Production microorganism	negative by test

*Analyzed at regular intervals

The heavy metals and lead specifications meet FCC and exceed JECFA requirements. The arsenic, total viable count and *E. coli* specs meet JECFA requirements and are not included in FCC. The total coliforms and *Salmonella* specs meet both FCC and JECFA. The antibiotic activity and mycotoxins specifications meet JECFA and are not included in FCC (although FCC mentions mycotoxins but has not established tolerances). The production microorganism specification is a Novo Nordisk specification and is not mentioned in FCC or JECFA.

6.3 Absence of the Production Strain

The manufacturing process includes a final germ filtration step to ensure that no production microorganism is present in the final enzyme concentrate. Absence of the production microorganism is part of the complete specification as outlined above.

000465



7. APPLICATION

7.1 Technological function

The active principle in Novozym 899 is the endo 1,4-beta-xylanase. The endo 1,4-beta-xylanase hydrolyses the beta-(1,4)-D-xylosidic linkages in the arabinoxylan backbone. Arabinoxylans, that may have been described as pentosans in previous literature, are highly branched xylans that are characteristic for the outer cell walls and endosperm of cereals such as wheat, barley, rye, and oat. The backbone is beta-(1,4) linked xylose which is mono or disubstituted with arabinose at the O-2 and/or O-3 position. Arabino-xylans exists both in a soluble and an insoluble form. Novozym 899 has activity towards both forms.

Wheat flour contains 2-4% arabinoxylans, which play an essential role in bread making. The soluble fraction (approx. 1/3 of the amount) is essential for the water retention capacity of flour. The insoluble fraction is coupled to proteins and is believed to reduce the elasticity of the gluten complex.

Novozyym 899 is used in baking to improve handling and stability of the dough. By its action on the insoluble fraction of the arabinoxylans in flour, the elasticity of the gluten network is increased.

Further information regarding Novozym 899 is given in the enclosed Product Sheet (App. C, ref. 3).

7.2 Use Levels

The enzyme preparation is used at minimum levels necessary to achieve the desired effect and according to requirements for normal production following cGMP.

The recommended dosage for Novozym 899 is 2-16 g per 100 kg flour corresponding to 50-400 FXU/kg flour.

7.3 Enzyme Residues in the Final Food

The enzyme is added to the flour and is active during the dough preparation and the leavening of the unbaked bread. During the baking process the high temperatures in the oven cause an inactivation of the enzyme activity.

000467



8. SAFETY EVALUATION

As stated most recently by FDA (App. A, ref. 12, 13), the issues relevant to a safety evaluation of an enzyme preparation are the safety of the enzyme source, the enzyme component, the manufacturing process, and a consideration of dietary exposure. Each of these is addressed below.

8.1 Production Strain

The safety of the production organism must be the prime consideration in assessing the probable degree of safety of an enzyme preparation intended for use in food (App. A, ref. 5). A safety evaluation of the genetically modified *F. venenatum* production strain based on the concepts initially outlined by Pariza and Foster in 1983 (App. A, ref. 5), and further developed by Berkowitz and Maryanski in 1989, IFBC in 1990, the EU SCF in 1991, the OECD in 1992, FAO/WHO in 1996, and ILSI Europe Novel Food Task Force in 1996, (App. A, ref. 6-11) demonstrates the safety of this genetically modified *F. venenatum* production strain. The components of this evaluation: the identity of the host strain, a description of the plasmids used, the sources and functions of the introduced genetic material, an outline of the genetic construction of the production strain, and some characteristics of the production strain are given in Section 3.

The host microorganism has a history of safe industrial use as 'Quorn' mycoprotein in human food (see Section 3.2), the genetic modifications are well characterized and specific, utilizing well-known plasmid and vector sequences, the introduced genetic material does not encode and express any known harmful or toxic substances (see Section 3.3-3.5), the introduced genetic material is stable and there is little, if any, potential for transfer of any of the introduced DNA sequences to other organisms (see Section 3.6), *F. venenatum* is not a plant or human pathogen (see Sections 3.7 and 3.8), and the production strain does not produce secondary metabolites of toxicological concern (see Section 3.9). Therefore, the genetically modified *Fusarium venenatum* LyMC4.B strain is considered a safe production strain for the xylanase enzyme preparation.

8.2 Enzyme Component

Enzyme proteins themselves do not generally raise safety concerns (App. A, ref. 5, 12, 18). The primary enzyme activity in Novozym 899 is xylanase, IUB EC 3.2.1.8, catalyzing the hydrolysis of xylosidic linkages in an arabinoxylan backbone. Xylanases, which are a types of pentosanases, are the most functional enzymes in the hemicellulase enzyme preparations, which are well known, in the baking industry (App. A, refs. 1-4).

As demonstrated in Section 4, the Novozym 899 enzyme preparation is substantially equivalent to the Pentopan Mono enzyme preparation. Identical xylanase genetic coding sequences were used in the construction of the production strains for Novozym 899 and Pentopan Mono. The *T. lanuginosus* xylanase coding sequence incorporated into the *F. venenatum* production strain has not been altered.



Several expert groups, as well as FDA and FDA scientists have discussed the concept of substantial equivalence relative to food safety assessment (App. A, ref. 6, 9-11, 19-22). Essentially all these groups conclude that if a food ingredient is substantially equivalent to an existing food ingredient known to be safe, then no further safety considerations other than those for the existing ingredient are necessary. In addition, FDA has applied this concept in the determination that several enzyme preparations are safe for use in food (App. A, ref. 20, 23, 24). In particular, differences in glycosylation between enzyme proteins was considered.

Novo Nordisk has previously determined that the Pentopan Mono xylanase enzyme preparation produced by *A. oryzae* expressing the xylanase from *T. lanuginosus* is GRAS. This conclusion and an overview of the information supporting the conclusion were presented to FDA in January of 1997. A letter from FDA to Novo Nordisk documents the presentation of this GRAS determination to FDA (App. C, ref. 1). In addition, an overview describing the safety evaluation of the *T. lanuginosus* xylanase expressed in *A. oryzae* and the conclusion that this xylanase enzyme is safe for use in baking applications has been published (App. A, ref. 25).

Based on the above considerations, the enzyme component, xylanase from *T. lanuginosus* is generally recognized as safe.

8.3 Manufacturing Process

Novozym 899 meets the specifications and requirements for enzyme preparations as outlined by FCC and JECFA (App. A, ref. 16, 17) (see Section 6). As described in Section 5, the xylanase enzyme preparation is produced in accordance with cGMPs, using ingredients that are accepted for general use in foods, and under conditions that ensure a controlled fermentation. These methods are based on generally available and accepted methods used for the production of microbial enzymes (App. D, ref. 1-3).

As explained in Section 5.2.4, the herbicide phosphinothricin-ammonium is used only in the inoculation medium, in a concentration of 5 g/l, in order to maintain the selection pressure on the introduced DNA sequences. It is concluded, based on a worst-case calculation, that the use in the inoculation flask may result in a maximum potential residue level in processed food that compares favorably to the 0.5 ppb threshold of regulatory concern level, established for substances used in food contact articles, 21 CFR §170.39.

A summary of the toxicological information available on phosphinothricin-ammonium was published in the Federal Register (App. D, ref. 4). In addition, the Environmental Protection Agency (EPA) established an exemption from the requirement of a tolerance for residues of the inert ingredients phosphinothricin acetyltransferase (PAT) and the genetic material, the *bar* gene, necessary for its production in 40 CFR §180.1151 (App. D, ref. 5). This is based on the conclusion that there is reasonable

certainty that no harm will result from aggregate exposure to the US population to the PAT protein and the *bar* gene as presented in 62 FR 17717-17720 (App. D, ref. 5). The OECD has also published a consensus document on general information concerning the *bar* gene and the phosphinothricin acetyltransferase protein that confer tolerance to phosphinothricin herbicide (App. D, ref. 6).

Based on this information it is concluded that the described use of phosphinothricin-ammonium in the inoculation medium is safe.



8.4 Safety Studies

The following studies were performed:

- 13 weeks oral toxicity study in rats
- Test for mutagenic activity (Ames Test)
- Human lymphocyte cytogenetic assay

A paper summarizing the safety studies and evaluating the safety in use of the xylanase enzyme preparation produced by the genetically modified *F. venenatum* has been accepted for publication (App. E, ref. 1). This paper concludes that there are no safety concerns when using this xylanase enzyme preparation from the selected strain of *F. venenatum* expressing the gene encoding the *T. lanuginosus* xylanase in food processing applications.

Three safety studies, a 13-week oral rat feeding study, a test for mutagenic activity, and a test for induction of chromosome aberrations were performed on the Novozym 899 batch PPQ 6125.

The main conclusions of the safety studies can be summarized as follows:

- Oral administration to rats of up to 10 ml/kg body weight/day (~ 89422 FXU/kg/day or 1.12 g TOS/kg/day) for 13 weeks reveals no signs of toxic effects related to treatment, and this dosage represents the No-Observed-Adverse-Effect-Level (NOAEL) in this study.
- Novozym 899 is not genotoxic, as no mutagenic activity was found in either Ames test or the human lymphocyte test.

All safety studies were performed with Novozym 899, batch PPQ 6125. This batch was obtained by mixing of 3 sub batches, each produced according to the description given in section 5, omitting stabilization and standardization. The composition of batch PPQ 6125 as determined by chemical analyses is given in Appendix E, reference 1.



8.5 Estimates of Human Consumption and Safety Margin

8.5.1 Estimates of human consumption

As stated in Section 7, the enzyme activity is largely inactivated during the baking process. However, in order to illustrate a "worst case" situation the following calculations are made assuming that all enzyme activity is retained in the bread.

Novozym 899 has an activity of 2500 FXU/g with an estimated organic solids content of 4% TOS.

The maximum recommended dosage of Novozym 899 is 16 g per 100 kg flour. Using a standard recipe, 100 kg flour results in 140 kg bread, giving a theoretical content of Novozym 899 of 114 mg/kg bread or 4.56 mg TOS/kg bread.

The average human intake of bread is estimated using well established statistics from various countries.

United Kingdom: The Ministry of Agriculture, Fisheries and Food: 1987 Annual Report of the National Food Survey Committee, Household Food Consumption and Expenditure:

Consumption of bread, cakes and biscuits per person per day is 158 g.

Denmark: "Levnedsmiddelstyrelsen": Development of Food Consumption in Denmark, 1955-1990, Description of the Danish Diet based on food statistics and nutrition calculated data:

Consumption of bread, flutes, pita bread, cakes, and rye bread per person per day is 123 g.

USA: Industrial Outlook 1992 (Food Beverages):

Consumption of bread and related products per person per day is 109 g.

In order to illustrate a "worst case" situation it is assumed that all bread and related products are produced using Novozym 899 as a processing aid.

Based on the highest daily intake of bread (158 g) the daily intake per person of Novozym 899 would be 18.1 mg corresponding to:
 $18.1 \text{ mg} \times 4\% \text{ TOS} = 0.7 \text{ mg TOS per day.}$

For an average person weighing 60 kg this corresponds to 1.2×10^{-5} g TOS per kg body weight per day.

8.5.2 Safety margin

The safety margin is calculated as dose level with no adverse effect divided by human consumption.

Assuming a NOAEL dose level in the 13 weeks oral toxicity study in rats is 10 ml/kg/day corresponding to 1.12 g TOS/kg/day.

The safety margin can thus be calculated to be:

For bread: $1.12 / 1.2 \times 10^{-5} = 9 \times 10^4$

8.6 Conclusion

The basic conclusions and guidelines for determining the safety of enzymes used in food processing as stated by Pariza and Foster (App. A, ref. 5) in 1983 and reiterated by FDA (App. A, ref. 21) can be applied to the Novozym 899 xylanase enzyme preparation. In particular, enzyme preparations derived, using cGMPs, from a non-pathogenic and non-toxigenic microorganism, which does not produce antibiotics, would not ordinarily present a basis for a safety concern and will be safe to consume at the low levels encountered in processed foods.

The information presented in the previous sections and the safety evaluation elaborated in this section demonstrate that Novozym 899, a xylanase enzyme preparation from a selected non-toxigenic strain of *Fusarium venenatum* expressing the gene encoding a xylanase from *Thermomyces lanuginosus*, meets these criteria and is safe for use in the production of bread.



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LIST OF APPENDICES

- APPENDIX A** General References
- APPENDIX B** Production Strain References
- APPENDIX C** Enzyme Identity and Characterization Information
- APPENDIX D** Manufacturing Process References
- APPENDIX E** Safety Reference



NOTE: *References and items listed in the appendices with a * before the number are included as attachments to this document.*

000474

APPENDIX A - General References

1. Olsen H.S.. *Enzymes in Food Processing, Biotechnology*, vol. 9, ed. by Rehm H.-J., and Reed G., (Weinheim: VCH), pp. 663-736, 1995.
2. Godfrey, T.. *Baking in Industrial Enzymology*, 2nd ed., Eds. Godfrey, T., and West, S., Chapter 2.5, pp87-101, 1996.
3. Sprösler, B.G., Xylanases in baking. *Proceedings from European Symposium on Enzymes and Grain Processing, Netherlands Dec 2-4 1996*. (Zeist, The Netherlands: TNO Nutrition and Food Research Institute). pp. 177-187, 1996.
4. Uhlig, H., *Industrial Enzymes and Their Applications*, John Wiley and Sons, Inc., 1998.
5. Pariza, M.W. and Foster, E.M.. Determining the Safety of Enzymes Used in Food Processing. *J. of Food Protection*, 46:5:453-468, 1983.
6. Berkowitz, D. and Maryanski, J.. Implications of biotechnology on international food standards and codes of practice. Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Eighteenth Session, Geneva, July 3-12, 1989.
7. IFBC (International Food Biotechnology Council). Chapter 4: Safety Evaluation of Foods and Food Ingredients Derived from Microorganisms in Biotechnologies and Food: Assuring the Safety of Foods Produced by Genetic Modification. *Regulatory Toxicology and Pharmacology* 12:S1-S196, 1990.
8. EU Scientific Committee for Food. Guidelines for the presentation of data on food enzymes. Reports of the Scientific Committee for Food, 27th series, 1991.
9. Organisation for Economic Cooperation and Development, *Safety Evaluation of Foods Derived by Modern Biotechnology*, 1993.
10. FAO/WHO. *Biotechnology and Food Safety, Report of a Joint FAO/WHO Consultation*. FAO FNP 61. Rome, Italy. 1996.
11. Jonas, D.A., Antignac, E., Antoine, J.M., Classen, H.G., Huggett, A., Knudsen, I., Mahler, J., Ockhuizen, T., Smith, M., Teuber, M., Walker, R., and de Vogel, P. The Safety Assessment of Novel Foods, Guidelines prepared by ILSI Europe Novel Food Task Force. *Food Chemical Toxicology* 34:931-940, 1996.
12. Food and Drug Administration. Lipase Enzyme Preparation From *Rhizopus niveus*: Affirmation of GRAS status as a Direct Food Ingredient. Fed. Regist. 63:24416-24419, 1998.
13. Food and Drug Administration. Carbohydrase and Protease Enzyme Preparations Derived From *Bacillus subtilis* or *Bacillus amyloliquefaciens*; Affirmation of GRAS Status as Direct Food Ingredients. Fed. Regist. 64:19887-19895, 1999.
14. Organisation for Economic Cooperation and Development, *Safety Considerations for Biotechnology*, 1992.



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000475

15. Becker, W.M., Kleinsmith, L.J., Hardin, J., World of the Cell, Benjamin Cummings Science, 2000.
16. Food Chemicals Codex, 4th Edition, National Academy of Sciences, Food and Nutrition Board, Committee on Food Chemicals Codex, National Academy Press, Washington, D.C., 1996.
17. Joint FAO/WHO Expert Committee on Food Additives, General Specifications for Enzyme Preparations Used in Food Processing, Comp. of Food Additive Specifications, Vol. 1, Annex 1, FAO, 1992.
18. Food and Drug Administration. Statement of Policy: Foods Derived From New Plant Varieties. Fed. Regist. 57:22984-23005, 1992.
19. Kessler, D.A., Taylor, M.R., Maryanski, J.H., Flamm, E.L., and Kahl, L.S. The Safety of Foods Developed by Biotechnology. Science 256:1747-1749, and 1832, 1992.
20. Food and Drug Administration. Enzyme preparations from animal and plant sources; Affirmation of GRAS status as direct food ingredients. Fed. Regist. 60:32904-32912, 1995.
21. Food and Drug Administration. Substances generally recognized as safe. Proposed Rule. Fed. Regist. 62:18938-18964, 1997.
22. Maryanski, J.H., Center for Food Safety and Applied Nutrition, US Food and Drug Administration. *FDA's Policy for Foods Developed by Biotechnology*, In *Genetically Modified Foods: Safety Issues*, Eds. Engel, Takeoka, and Teranishi, American Chemical Society, Symp. Series No. 605, Ch. 2, pp. 12-22, 1995.
23. Food and Drug Administration. Microbially derived chymosin enzyme preparations: Affirmation of the GRAS status. Fed. Regist. 55:10932-10935, 1990. Fed. Regist. 57:6476-6479, 1992. Fed. Regist. 58:27197-27202, 1993.
24. Food and Drug Administration. Secondary Direct Food Additives Permitted in Food For Human Consumption: Milk-Clotting Enzymes. Fed. Regist. 62:59281-59284, 1997.
25. Bergman, A. and Broadmeadow, A., An overview of the safety evaluation of the *Thermomyces lanuginosus* xylanase enzyme (SP628) and the *Aspergillus aculeatus* xylanase enzyme (SP578). Food Add. and Contam. 14:389-398, 1997.



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APPENDIX B - Production Strain References

1. Nirenberg, H.. Morphological differentiation of *Fusarium sambucinum* Fuckel sensu stricto, *F. torulosum* (Berk. & Curt.) Nirenberg comb. nov. and *F. venenatum* Nirenberg sp. nov. Mycopath. 129:131-141, 1995.
2. Weibe, M.G. et al, 1991. Appearance of morphological (colonial) mutants in glucose-limited, continuous flow cultures of *Fusarium graminearum* A3/5. Mycol. Res., 95 (11):1284-1288.
3. Yoder, W.T. and Christianson, L.M., 1998. Species-Specific Primers Resolve Members of *Fusarium* Section *Fusarium*. Taxonomic Status Of the Edible "Quorn" Fungus Reevaluated. Fungal Genetics and Biology, 23: 69-80.
4. UK Ministry of Agriculture, Fisheries and Food, 1985, Certificate of Free Sale for 'Quorn' Mycoprotein.
5. Trinci, A.P.J., 1992. Myco-protein: A twenty-year overnight success story. Mycol. Res. 96:1-13.
- * 6. Identification of two *Fusarium* cultures, Dr. H. Nirenberg, 1997-02-21. Novo Nordisk memo, Identification of two *Fusarium* cultures by Dr. Helgard Nirenberg, Luna#: 2000-06099-01.
- * 7. Identification and characterization of *Fusarium* strain ATCC 20334. Prof. Ulf Thrane, the Danish Technical University, 1997-02-19.
8. O'Donnell, K. et al, 1998. Molecular Phylogenetic, Morphological, and Mycotoxin Data Support Reidentification of the Quorn Mycoprotein Fungus as *Fusarium venenatum*. Fungal Genetics & Biology, 23:57-67.
9. *Fusarium* Species. An Illustrated Manual for Identification. P.E. Nelson, T.A. Toussoun and W.F.O. Marasas, The Pennsylvania State University Press, pp142-145, 1983.
10. Royer, et al. 1995. *Fusarium graminearum* A 3/5 as a Novel Host for Heterologous Protein Production. BIO/TECHNOLOGY 13:1479-1483.
11. Thompson et al, 1987. Characterization of the herbicide - resistance gene bar from *Streptomyces hygroscopicus*. EMBO (Eur Mol Biol Org) J. 6 (9): 2519-2523).
12. Corrick, C. M., A. P. Twomey, and M. J. Hynes. The nucleotide sequence of the *amdS* gene of *Aspergillus nidulans* and the molecular characterization of 5' mutations. Gene 53:63-71. 1987.
13. Boel, E. et al. 1984. Two Different Types of Intervening Sequences in the Glucoamylase Gene from *Aspergillus niger*. EMBO 3:1581-1585.

14. Yanish-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* 33:103-119.
15. Donald G. Cooney and Ralph Emerson, *Thermophilic Fungi*, ed. W. H. Freeman and Company, 1964, pp. 82-87.
- * 16. Taxonomy of *Thermomyces lanuginosus*, internal Novo Nordisk memorandum, May 13, 1996.
17. Royer, J.C. et al, 1999. Deletion of the Trichodiene Synthase Gene of the Fungus *Fusarium venenatum*; Two Systems for Repeated Gene Deletions. *Fungal Genetics and Biology*, 28: 68-78.
- * 18. *Fusarium venenatum*. Human Pathogenic Potential. Novo Nordisk report, 1999-08-03.
19. Food and Drug Administration. Ranks, Hovis, McDougall Research, Ltd.; Filing of Food Additive Petition. Fed. Regist. 51:19610, 1986.
- * 20. Miller, J. D. and MacKenzie, S., 2000. Secondary metabolites of *Fusarium venenatum* strains with deletions in the Tri5 gene encoding trichodiene synthetase. *Mycologia*. 92:764-771.
- * 21. Pedersen, P. B. and Miller, J. D., 2000. The fungal metabolite Culmorin and related compounds. Natural toxins. In Press.
- * 22. Enniatins. Novo Nordisk report, 1999-08-16.
- * 23. Fusarin data in literature. Novo Nordisk report, 1999-10-05.
- * 24. Toxicity of the Secondary Metabolite Fusarin C produced by *Fusarium* spp. A literature survey. Novo Nordisk report, 1999-11-30.



000480

To: PHVa

From: WTY

Date: July 6th, 2000

Luna #: 2000-06099-01



Novo Nordisk

1

MEMO

**Identification of two *Fusarium* cultures by Dr. Helgard Nirenberg
(1997-02-21)**

Summary

This report summarizes the identifications made by Dr. Helgard Nirenberg (Federal Biological Research Center for Agriculture and Forestry, Institute of Microbiology, Berlin, Germany) of two *Fusarium* cultures sent to her, by WTY at NNBT, in November and December of 1996. Dr. Nirenberg is internationally respected and recognized as one of the leading *Fusarium* taxonomists worldwide. The cultures were sent to her "Blind" so as not to bias the outcome of her identification.

Strains

(1) The first strain (ATCC¹ 20334) was sent to Dr. Nirenberg as an "unidentified" *Fusarium* species on November 21st, 1996 and arrived on November 25th. This strain (the "first culture" in Dr. H Nirenberg's report, dated 1997-02-21) was described as being degenerated with little yellowish aerial mycelium.

Dr. Nirenberg identified this strain as *Fusarium venenatum*.

(2) The second strain (ATCC 60879) had been identified as *Fusarium crookwellense*(=*F. cerealis*) by the ATCC but had subsequently been found (by WTY at NNBT) to generate identical RAPD² banding patterns to ATCC 20334, which were different from other *F. crookwellense*/*F. cerealis* RAPD banding patterns.

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This strain was sent (again, as an "unidentified" *Fusarium* species) to Dr. Nirenberg as a "control" for the ATCC 20334 strain. It was mailed from NNBT on December 5th and arrived on December 9th. This strain was described in Dr. H. Nirenberg's report (dated 1997-02-21) as an isolate producing reddish aerial mycelium.

Dr. Nirenberg identified the second strain as *Fusarium venenatum*.

Conclusion

Dr. Nirenberg identified both strains as *Fusarium venenatum*

-
1. ATCC: American Type Culture Collection
 2. RAPD: Random Amplified Polymorphic DNA

000481.001



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Your ref.letter from

Our reference
MB/Dr. Nii/et

Telephone Date
8304-2210/-2211 Feb 21, 1997

Identification of two *Fusarium* cultures

Both cultures which arrived at the 25th of November and at the 9th of December 1996 belong to the species *F. venenatum*.

The first culture was quite degenerated with little yellowish aerial mycelium but nevertheless it exhibited the typical characteristics of this species: It produces chlamydospores laterally and often in clusters, the sporodochial conidia were > 6 µm wide, strongly bent with a pronounced footcell. Both features are not found in *F. culmorum* which is morphologically the closest species.

The other isolate produced still a lot of reddish aerial mycelium. The chlamydospores were produced laterally and intercalary in clusters, the sporodochial conidia were ca. 7 µm wide, strongly bent and also with a pronounced footcell.

The price of the identification will be 150,- DM per culture. The bill for 300,- DM will be sent by separate mail by our administration.

Sincerely

Dr. H. Nirenberg

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Appendix
B-7



NOVO NORDISK A/S
Novo Allé
2800 BAGSVÆRD

att. Henrik Møllgaard
att. Ib Groth Clausen

→

AM 97 02 24

F-97 00534

Hch

cc: AKM, WTY, GRAM, EJB,
SC, LLa, IGC, HMay,

→ ARK!

Lyngby, 19. februar 1997

Identification and characterization of *Fusarium* strain ATCC 20334.

The strain, ATCC 20334, was delivered by NOVO NORDISK A/S. The result of my examination can be summarized as:

ATCC 20334 is identified to be:

Fusarium venenatum Nirenberg 1995

based on conidial morphology, growth rates on PSA and TAN, and the lobed colony margin on PSA.

No known secondary metabolites could be detected, by HPLC and TLC of culture extracts.

By TLC analysis the extract of ATCC 20334 showed few metabolites; however, they could all be found in extracts of *F. venenatum* IBT 1154, 1155, 1170, 1172, 1173 analyzed on the same TLC plate. This supports the identity of ATCC 20334.

Fusarium venenatum was described by Nirenberg (1995), Mycopathologia 129(3):131-141, and physiological and chemical characterization is published by Thrane & Hansen (1995), Mycopathologia 129(3): 183-190 and Altomare et al. (1995), Mycopathologia 129(3):177-181. By a typesetting error *F. venenatum* is misspelled as '*F. venenatum*' in the latter two papers.



Experimental details:

The strain was cultured on the following agar substrates:

SNA = Spezieller Nährstoffarmer Agar (morphology)
PSA = Potato Sucrose Agar (growth rate and pigmentation)
TAN = Tannin Sucrose Agar (growth rate)

For HPLC analysis the strain was cultured on:

Potato Sucrose Agar
Yeast Extract Sucrose Agar (yeast extract from Difco)
Yeast Extract Sucrose Agar (yeast extract from Sigma, Y-4000)
Rice Meal Agar

Three 9 cm Petri dishes of each substrate were inoculated and incubated for 14 days at 25°C in the dark. The 12 cultures were extracted by 75 ml chloroform/methanol (2/1) followed by a second extraction using 75 ml ethyl acetate + 1 ml formic acid. The organic phases were pooled, evaporated to dryness, the residue was taken up in 2 ml of methanol and defatted with light petroleum. 10 µl was injected for the analysis by HPLC with diode array detection (J. Chromatogr. Library 54: 253-372, 1993).

The print-outs of HPLC analyses acquired on 3 DEC 96 (HP1090M "Obelix") and 8 JAN 97 (HP1100 "Idefix"), respectively, are enclosed.

Sincerely yours

Ulf Thrane
Assoc.Prof., Ph.D.

000485

000486

To: PE
From: KMO
Copy: MiSa, IGC, HDA

Taxonomy of *Thermomyces lanuginosus*

In relation to a filing to FDA, PE has requested documentation in support of the change of species name from *Humicola lanuginosa* (Griffon and Maublanc) Bunce to *Thermomyces lanuginosus* Tsiklinsky.

This memo is an attempt to summarize the results of a literature search for such data.

The generic name *Thermomyces* was introduced by Tsiklinsky in 1899 for one species, *T. lanuginosus*. The species was described and photographs taken to illustrate the morphology. No type specimen was deposited.

The species as described by Tsiklinsky is very characteristic morphologically and physiologically and there is little doubt that Tsiklinsky's *Thermomyces lanuginosus* is the same as isolated and described by later workers (such as for instance Bunce (1961) and Cooney & Emerson (1964)) as *Humicola lanuginosa*.

According to The International Code of Botanical Nomenclature the correct name is the earliest legitimate one. Thus *Thermomyces lanuginosus* should be the correct name, provided it is legitimate. However due to Tsiklinsky's rather incomplete description of the species this legitimacy has been questioned by many mycologists. Thus the main reason for the taxonomical confusion relates to different views upon whether Tsiklinsky's description meets the requirements for a valid publication.

The decision to favour the name *Thermomyces* at Novo Nordisk is based upon the following circumstances:

- All major commercial culture collections (ATCC: American Type Culture Collection, IMI: International Mycological Institute, CBS: Centraalbureau voor Schimmelcultures) now use the name *T. lanuginosus*.
- A neotype named *T. lanuginosus* has been selected at IMI (IMI 84400)
- Most recent publications discussing this problem are in favour of using the name *T. lanuginosus*:

Pugh G.J.F. et al (1964) *Trans. Brit. mycol. Soc.* 47(1), 115-

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Fassatiova O. (1967) Česká Mykologie 21, 78-89
Manoch L. et al. (1986) Trans. Brit. mycol. Soc. Japan

However the name *Humicola lanuginosa* is still seen in literature. The extended use of this name is probably due to the popularity of the book of Cooney & Emerson (1964) "The Thermophilic Fungi", which recommends the use of the name *Humicola lanuginosa*.

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APPENDIX

B-18

Appendix B-18

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Enzyme Business Toxicology

Date : 1999-08-03
File : 1999-07137-01
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Fusarium venenatum
Human Pathogenic Potential

CONTENTS

	PAGE
1. INTRODUCTION	2
2. TAXONOMY	2
3. HUMAN INFECTIONS ASSOCIATED WITH FUSARIUM SPECIES	3
4. CONCLUSION	3
5. REFERENCES	3
LAST PAGE	4

Author :
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Issued by :
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1. Introduction

Hyphomycetes, like species of the genus *Fusarium*, are generally not regarded as primary human pathogens. They are adapted to a living as saprophytes and many of them as plant parasites, and fungal spores or hyphal fragments which are deposited on the skin or enter the body via one of its orifices are generally incapable of further growth and development.

Exceptions to this are a few dimorphic fungi which readily convert to a yeast-like growth phase at 37°C in vivo as well as in vitro. These are the true pathogenic fungi, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis* and *Sporothrix scneckii* (Ref. 3-8).

Some soil saprophytes are to some extent able to adapt to and proliferate in the abnormal environment of mammalian tissue if they are inhaled or traumatically implanted. They are often described as the etiologic agent of different mycosis. A well known example of this category is *Aspergillus fumigatus*, as the primary agent of aspergillosis.

However, the ability to growth at body temperatures does convey on fungi a pathogenic potential which can be expressed if the fungus propagules are in exceptionally high concentrations and if the host is suitable compromised, that is when natural immunity is decreased by other diseases serious wounds or during treatment with drugs such as immunosuppressants, chemotherapeutics, or antibiotics. They adapt to and take advantage of the presented organic substrate, the compromised patient. Such fungi and the diseases they cause are thus termed opportunistic (Ref.5).

This report is concerned with the human safety of *Fusarium venenatum* as a part of the safety assessment of strains within this species intended for enzyme production. It deals with the potential pathogenicity of *F. venenatum* and the opportunistic infections reported in the literature from strains of this species and related species within the genus *Fusarium*.

2. Taxonomy

The taxonomy of the genus *Fusarium* have been a subject of controversy for many years. And a crucial problem encountered when working with *Fusarium* strains is the correct identification of each strain. This is essential when evaluating the pathogenic potential of a strain based on the literature.

The genus *Fusarium* belongs to the class of imperfect fungi *Hyphomycetes* (*Moniliales*). *Fusarium* species are very common saprophytes on plant debris and in soil and world wide in distribution. Many species are among the most important plant pathogens in the world.

Fusarium venenatum is placed in the *Discolor* section of this genus together with species like *Fusarium graminearum*, *Fusarium sambucinum*, *F. sulphureum* and *F. cerealis* (*F. crookwellense*) (ref.9,10).

Fusarium sambucinum Fuckel (= *F. roseum* Link) (Teleomorph: *Gibberella pulicaris*, Ascomycetes/Sphaeriales) is type species for the genus and therefore of special significans for *Fusarium* taxonomists. Recently the taxonomy of this species has been evaluated in "The European *Fusarium sambucinum* project" (ref.11). Based on traditionally morphological criteria Nirenberg (ref.12) divided the *F. sambucinum* complex (sensu lato) into three different species: *F. sambucinum* (sensu stricto) and two new species *F. torulosum* and *F. venenatum*. Modern methods of molecular biology, serology and metabolite profiles confirmed this distinction in three different phylogenetic entities (ref.11). It should be noticed that several synonyms for *F. sambucinum* (sensu lato) have been applied in the past, and these species designations have not been included in the present literature search, except from *F. sulphureum* which have been widely used (nomen dubium?). The species designation *F. venenatum* apparently appears for the first time in the European *Fusarium sambucinum* Project (EFSP) (ref.11) and all available references to this species are contained in the reports published from this project. No obvious and specific synonyms are deducible from the literature. Nirenberg states that "*F. sulphureum* might be synonymous with either *F. cerealis* or *F. venenatum*", but also *F. culmorum* var. *cerealis* and *F. sambucinum* var. *coeruleum* are mentioned.

F. cerealis is a rather new species. Apparently it was separated from *F. culmorum* var. *cerealis* in the early eighties and given the name *F. crookwellense* and only recently renamed as *F. cerealis*. Both names have been included in this investigation.

3. Human infections associated with Fusarium species

An increasingly number of report have been published connecting Fusarium species with opportunistic mycosis in humans. These infections predominantly occur in the nails, onychomycosis, and in the cornea, mycotic keratitis. But Fusarium species have also been hold responsible for causing ulcers, necrosis, and other lesions of the skin in addition to disseminated mycosis and infections in some organs and tissues in patients with severe underlying diseases. A number of general reviews concerning these opportunistic Fusarium infections of man and other animals have been written (Ref. 13-15).

Fusarium species most often reported from human infections have been *F. solani*, *F. oxysporum*, and *F. moniliforme*, in decreasing order. Only a limited number of other species like *Fusarium proliferatum*, *F. chlamyosporum*, *F. dimerum*, *F. sacchari*, *F. semitectum* and *F. nivale* have been reported and only rarely.

No one of these species described in opportunistic infections belong to the Discolor-section.

The species so far reported from human and animal infections have no apparent morphological or physiological features in common which might explain their pathogenicity and as yet no apparent adaptation to an animal host has emerged. Their temperature optima and often maxima are below 37°C but the mammalian skin surface may be only at 33°C. Inability to withstand desiccation is a feature of *Fusarium* hyphae and a very high water activity in the substrate is a requirement for almost all species, which partly may explain why some *Fusarium* species play a minor role in human and animal mycosis (Ref.13).

The role of *F. solani* and *F. oxysporum* in eye infections may demand greater attention because these infections seems for unknown reasons only related to these two species and they account for a large part of the reports on *Fusarium* infections in humans.

4. CONCLUSION

We have found no reports connecting *Fusarium venenatum*, *Fusarium sambucinum* or apparently any species within the Discolor-section with human or animal infections.

5. REFERENCER

1. Joffe, A.Z. ed. (1986): *Fusarium species: Their Biology and Toxicology*. John Wiley & Sons.
2. Chelkowski, J. ed. (1989): *Fusarium. Mycotoxins, Taxonomy and Pathogenicity. Topics in Secondary Metabolism 2*. Elsevier.
3. Rippon, J.W. (1988): *Medical Mycology. The Pathogenic Fungi and the Pathogenic Actinomycetes*. W.B. Saunders Company.
4. Kwon-Chung og Bennett (1992): *Medical Mycology*. Lea & Fibiger.
5. Smith, J.M.B. (1989): *Opportunistic Mycoses of man and other Animals*. C.A.B. International Institute.
6. Gorbach, S.L. et al ed. (1992): *Infectious Diseases*. W.B. Saunders Company.
7. Howard, B.J. et al (1987): *Clinical and Pathogenic Microbiology*. The C.V. Mosby Company.
8. Balows, A. et al. (1991): *Manual of Clinical Microbiology*, 5 edition. American Society of Microbiology, Washington.

9. Gertach,W. and Nirenberg,H (1982): The Genus *Fusarium*: A Pictorial Atlas. Mitt. Biol. Bundesanst.Land-Forstw. Berlin.
10. Nelson,P.E. and al. (1983): *Fusarium* species: An illustrated manual for identification. University Park/London: Pennsylvania State University Press.
11. Nirenberg,H.I. ed. (1995): The European *Fusarium sambucinum* project. In: *Mycopathologia* Vol 129, No.3. Kluwer Academic Publishers.
12. Nirenberg,H.I.(1995). Morphological differentiation of *Fusarium sambucinum* Fuckel sensu stricto, *F. torulosum* (Berk.& Curt.) Nirenberg comb.nov. and *F. venenatum* Nirenberg sp.nov. In: *The European Fusarium sambucinum* project. *Mycopathologia* Vol 129, No.3. 131-141.Kluwer Academic Publishers.
13. Austwick,PKC (1984) *Fusarium* infections in man and animals. In, *The Applied Mycology of Fusarium*. Symposium of the British Mycological Society held at Queen Mary College, London, September 1982, edited by Moss, MO & Smith, JE, Cambridge University Press, UK.
14. Summerbell RC, et al. (1988): *Fusarium proliferatum* as an agent of disseminated infection in an immunosuppressed patient. *Journal of Clinical Microbiology* 26:82-87.
15. Joffe (1989): Human Infections associated with *Fusarium* Species. In: *Fusarium* species: Their Biology and Toxicology. John Wiley & Sons.p.293-298

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14 April, 2000

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Dear Dr. Pedersen

Re: The fungal metabolite culmorin and related compounds

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The fungal metabolite culmorin and related compounds

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1999-10009-01

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ABSTRACT

This paper reviews the toxicology of culmorins, a family of compounds found in grains contaminated by *Fusarium graminearum* and related fungi. We include the results of an Ames test and studies based on Quantitative Structure-Activity Relationships. Culmorin has low toxicity in several in vitro assays and in one study in swine and is Ames test negative. Culmorin is moderately antifungal. QSAR analysis suggested that the plant compound longifolene was similar. Longifolene is a GRAS compound used in cosmetics and is also moderately antifungal.

Key words: culmorin, Ames test, toxicity, longifolene

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INTRODUCTION

The sesquiterpene diol culmorin (1; $C_{15}H_{26}O_2$, molecular weight 238.4) was first isolated by Ashley et al. (1937) from *Fusarium culmorum* (WG Smith) Sacc followed by characterization some 30 years later by Barton and Westiuk (1967). It has been since shown to be produced by *F. graminearum* Schwabe, *F. crookwellense* Burgess, Nelson & Toussoun, and *F. venenatum* Nirenberg (Greenhalgh et al., 1984; Lauren et al., 1992; Miller & MacKenzie, 1999). All four species have been shown to produce related compounds including 5 OH-culmorin (2), culmorone (3) and 15 OH-culmorone (4; Kasitu et al., 1992; Miller & Mackenzie, 1999). These compounds have been detected in grains infected by *F. graminearum* and *F. culmorum* (Foster et al. 1986; Langseth et al. 1998). Culmorin has also been isolated from the lignicolous marine Ascomycete *Leptosphaeria oraemaris* Linder and detected in contaminated wood (Strongman et al., 1987). Another marine fungus, *Kallichroma tethys* Kohlm & Volkm Kohl produces isoculmorin (Alam et al., 1996). The detection of culmorin and related metabolites in grain samples in amounts similar to the trichothecenes present (Langseth et al. 1998) prompts some consideration of the toxicity of these compounds.

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Figure 1. Structures of compounds discussed in this paper.

1 culmorin

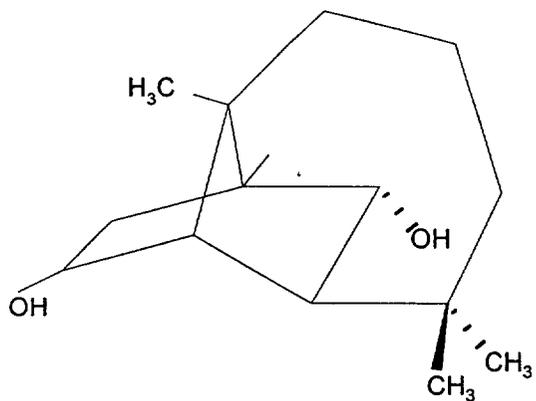
2 5 hydroxy cumorin

3 culmorone

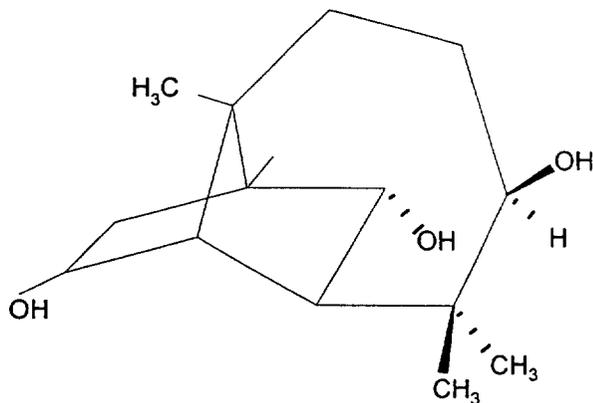
4 15 hydroxyculmorone

5 longifolene

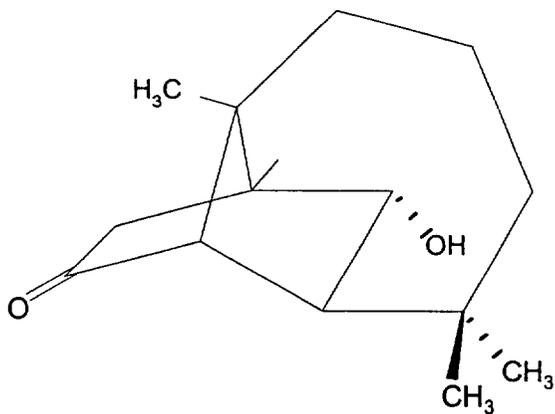
6 photodieldrin



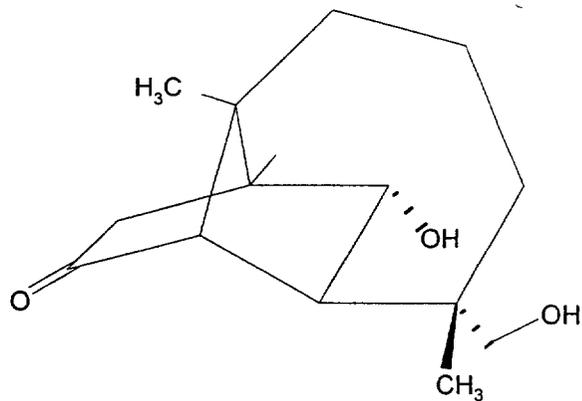
1



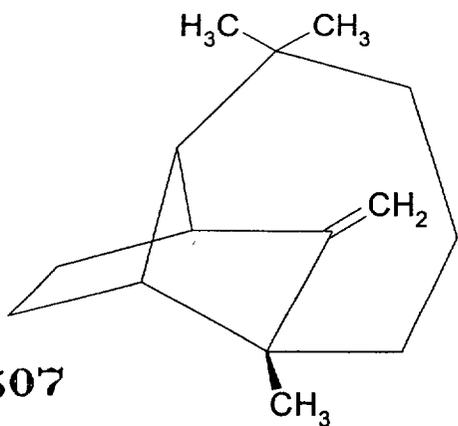
2



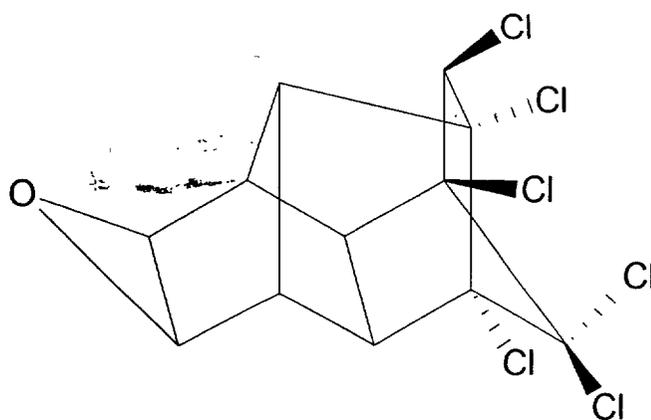
3



4



5



6

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MATERIALS AND METHODS

A bacterial reverse mutation assay was carried out to determine the ability of the fungal metabolite culmorin to induce gene mutations in bacteria and conducted in general accordance with OECD Guidelines for testing of chemicals, No. 471 and current GLP regulations (Anon. 1996; 1997). Culmorin (>99% pure) was isolated from *F. culmorum* HLX 1503. It was dissolved and diluted in ethanol. Four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) and *Escherichia coli* (WP2uvrA) were used. These strains were obtained from Dr. Bruce Ames, University of California at Berkeley and The National Collection of Industrial and Marine Bacteria Ltd. Aberdeen, Scotland, respectively. They were stored as frozen stock cultures. The correct genotypes of all bacterial test strains used were verified as described by Maron and Ames (1983) and Green (1984).

An inoculum from a frozen stock culture was grown overnight at approximately 37° C in Bacto Nutrient Broth (Difco). The minimal medium plates for the selection of histidine and tryptophan revertants consisted of the Vogel-Bonner medium E with 2% glucose and 1.5% Bacto agar. The overlayer agar contained the following ingredients per 100 ml volume according to Maron and Ames (1983): 0.6 g of purified agar, 0.5 g NaCl, and 10 ml of a 0.5 mM L-histidine/0.5 mM biotin solution (*Salmonella* strains) or 10 ml of a 0.5 mM tryptophan (*E. coli* strain). A S9 microsome fraction (9000 x g supernatant) prepared from adult male Sprague Dawley rat livers induced with Aroclor 1254 was obtained from Life Science Denmark Aps (Copenhagen).

The plate incorporation assay was essentially performed as described by Ames et al. (1975) and Maron and Ames (1983). Bacteria were exposed to 6 doses separated with bi-sections and with

2.5 $\mu\text{mol}/\text{plate}$ as the highest dose level applied. Two ml molten top agar at 45 C were added to 0.1 ml bacterial culture, 0.1 ml of each dose of culmorin or solvent (ethanol) or positive control substance and 0.5 ml S-9 mix (10% v/v) or 0.5 ml phosphate buffer. After mixing the soft agar was poured onto the selective agar plates (3 replica plates per dose). Revertant colonies were counted about 64 h after incubation at 37° C.

An analysis of the structural alerts of culmorin was made using two software packages. The first used Oxford Molecular's RS3 for Discovery of a database of genotox test results. The database was compiled from the open literature, and has about 60,000 test results on 16,000 chemicals in the database of Proctor & Gamble (Cincinnati, OH). The second used several modules of the TOPKAT™ system including NTP cancer bioassay, reproductive toxicology, rat oral LD₅₀ and Ames test (Oxford Molecular Group, UK).

RESULTS & DISCUSSION

The results of the Ames test are presented in Table 1. The sensitivity of the individual bacterial strains and the activity of the S9 mix was demonstrated under similar conditions to those used to test culmorin. No evidence of toxicity was observed following treatment with culmorin, but an apparently weak inhibition was observed at the highest dose (data not shown). Culmorin did not induce gene mutations in bacteria when tested to a concentration of 2.5 $\mu\text{mol}/\text{plate}$ in either the presence or absence of S9. To our knowledge, this is the first Ames test reported on culmorin.

It is believed that it was the antifungal activity of culmorin that stimulated interest in this compound in the years after its discovery. Strongman et al. (1987) showed the antifungal activity

of culmorin against a variety of marine and medically-relevant fungi as being in the 1 μM range. Culmorin was also phytotoxic to wheat coleoptile tissue at 0.1 to 1 μM , depending on cultivar (Wang and Miller, 1988).

The only reported study on the toxicity of culmorin in mammals was published by Rotter et al. (1992). They examined different *F. graminearum/culmorum* metabolites and their interaction with deoxynivalenol (DON) in growing piglets. Five pure-bred Yorkshire barrows (12-15 wk, 22.8 kg in average) were feed ad libitum with a diet containing 2 mg culmorin per kg without and in combination with 6 mg DON per kg feed for 21 days. These concentrations are similar to those that can occur in nature. Feed consumption and weight gain during the experiment as well as organ weights and appearance including stomach (colour, thickness and inflammation) at necropsy were determined. At ca. 0.2 mg/kg BW, no significant effects of culmorin alone or in combination were observed. Culmorin alone stimulated the feed consumption and weight gain slightly which might have become significant had more animals been used in the study.

Dowd et al. (1988) fed a similar list of *F. graminearum/culmorum* metabolites to two insects associated with maize *Heliothis zea* Boddie and *Spodoptera frugiperda* JE Smith to determine their effects and interaction with DON. Culmorin at 0.1 μM in the diet had no effect on growth and reproduction. In a treatment comprising 0.4 μM culmorin in combination with 0.8 μM DON in the diet, there was a significant synergism for both mortality and reduced growth in *H. zea*, an effect not seen in *S. frugiperda*.

The LD_{50} for culmorin in the chick embryotoxicity test (CHEST) was between 68.0 and 78.2

µg/egg (Prelusky et al. (1989). The investigators found a high correlation ($r = 0.729$, $P < 0.05$) between the CHEST-bioassay (µg/egg) and mouse LD₅₀ (ip) data (mg/kg BW). For 75% of the tested metabolites with available mouse data this ratio fell within the range 4.4-12.4. Based on this correlation, the mouse LD₅₀ (ip) would be in the range 250-1000 mg/kg BW indicating a low mammalian toxicity. The cytotoxicity of culmorin in BK-1 cells using the MTT cleavage test was in the 0.1 µM range, ca. 7-10 times less cytotoxic than DON in the same trial (Miller and Mackenzie, 1999).

The Ames mutagenicity, NTP carcinogenicity call and chronic LOAEL TOPKAT™ models predicted negative results. However, these predictions were not valid because they were outside the acceptable statistical envelope. The TOPKAT™ rat oral LD₅₀ model produced a statistically valid prediction of 1.3 g/kg, indicating very low toxicity.

The structural relational database identified two compounds as possibly being similar to culmorin. One was the natural compound longifolene (5, CAS 475-20-75), which is found in orange peel oil, lignonberry, globe artichoke, carrot seed, laurel and turpentine oil. The rat oral LD₅₀ of longifolene compound exceeded 5g/kg. It has been tested on the skin of human volunteers and it may be irritating to eyes and skin (Ford et al., 1992). Toxicity data exist for rats, rabbits, guinea pigs, fish, brine shrimp and bacteria including the Microtox (R) all showing low or no toxicity (e.g. Moreno, 1977; Sweet, 1997). Longifolene has been used in cosmetics and is generally regarded as safe.

The other compound flagged from the structural relational database was photodieldrin (6; CAS

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13366-73-9). Photodieldrin has six chlorine atoms and hence has a very different structure than culmorin (1). However, it is not carcinogenic in male or female mice and rats but is positive in the Ames test (Ashby and Tennant, 1988).

Culmorin has been found in experimentally-contaminated maize also containing DON both at ca. 25 mg/kg (Foster et al., 1986) and in naturally-contaminated small grains again in amounts that are comparable to the DON present (Langseth et al. 1998). Based on toxicology studies in swine, cultured cells, the CHEST assay, the results of the Ames test reported here, there is little evidence that culmorin and similar compounds have material toxicity. Further analysis based on the best available QSAR computer based modeling, culmorin can be compared to the Generally Regarded As Safe (GRAS) compound longifolene.

As noted above, culmorin has antifungal activity, a property also shared by longifolene (Krupa and Nylund, 1972; Nandi and Fries, 1976). It is known that grains experimentally-infected with *F. graminearum* have few other fungi present (e.g. Miller et al., 1983). Perhaps culmorin mediates this phenomenon.

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REFERENCES

Alam M, Jones EBG, Hossain MB, van der Helm D (1996): Isolation and structure of isoculmorin from the marine fungus *Kallichrona tethys*. J Natural Products 59:454-456.

Ames BN, McCann J, Yamasaki E (1975): Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutation Research 31:347-364.

Anon. (1996): European Agency for the Evaluation of Medical Products, 1996. ICH Topic S 2 A. Genotoxicity Tests for Pharmaceuticals. ICH Harmonised Tripartite Guideline.

Anon. (1997): OECD principles of Good Laboratory Practice (GLP), ENV/MC/CHEM(98)17. OECD. Genetic Toxicology: Bacterial Reverse Mutation Test. In OECD Guidelines for testing of chemicals. OECD Paris, Test Guideline 471. Revised document 1997.

Ashby J, Tennant RW (1988): Chemical structure, *Salmonella* mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogenesis among 222 chemicals tested in rodents by the U.S. NCI/NTO. Mutation Research 204:17-115.

Ashley JN, Hobbs BC, Raistrick H (1937): *LV*. Studies in the biochemistry of micro-organisms. *LIII*. The crystalline colouring matters of *Fusarium culmorum* (WG Smith) Sacc and related forms. Biochem J 31:385-397.

000513

Barton DHR, Werstiuk NH (1967): The constitution and stereochemistry of culmorin. *J Chem Soc Chem Commun* 1:30-31.

Dowd PF, Miller JD, Greenhalgh R (1989): Toxicity and interactions of some *Fusarium graminearum* metabolites to caterpillars. *Mycologia* 81:646-650.

Ford RA, Api AM, Letizia CS (1992). Monographs on fragrance raw materials. *Food Chemical Toxicology* 30 (s):1-138.

Foster BC, Neish GA, Lauren DR, Trenholm HL, Prelusky DB, Hamilton RMG (1986) Fungal and mycotoxin content of slashed corn. *Microbiologie Aliments Nutrition* 4:199-203.

Green MHL (1984): Mutagen testing using Trp⁺ reversion in *Escherichia coli*. In: *Handbook of Mutagenicity Test Procedures*, second edition. Edited by B. J. Kilbey et al. (Elsevier), pp. 161-187.

Greenhalgh R, Meirer RM, Blackweel BA, Miller JD, Taylor A, ApSimon JW (1984): Minor metabolites of *Fusarium roseum* ATCC-28114. *J Agric Food Chem* 32:1261-1264.

Kasitu GC, ApSimon JW, Blackwell BA, Fielder DA, Greenhalgh R, Miller JD (1992): Isolation and characterization of culmorin derivatives produced by *Fusarium culmorum* CMI 14764. *Can J Chem* 70:1308-1316.

000514

Krupa S, Yland JE (1972) Studies on ectomycorrhizae of pine. Growth inhibition of two root pathogenic fungi by volatile organic constituents of ectomycorrhizal root systems of *Pinus sylvestris*. European J Forest Pathology 2:88-94.

Lauren DR, Ashley A, Blackwell BA, Greenhalgh R, Miller JD, Neish GA (1992): Trichothecenes produced by *Fusarium crookwellense* DAOM 193611. J Agric Food Chem 35:884-889.

Langseth W, Ghebremeskel M, Kosiak B (1998): The occurrence of culmorin and hydroxyculmorins in cultures of *Fusarium culmorum* and *Fusarium graminearum* and naturally infected cereals. COST Action-835: Agriculturally important toxigenic fungi. EU Workshop, Athens 29.-31. Oct. 1998.

Maron DM, Ames BN (1983): Revised methods for the *Salmonella* mutagenicity test. Mutation Research 113:173-215.

Miller JD, Young JC, Trenholm HL (1983) *Fusarium* toxins in field corn. I. Parameters associated with fungal growth and production of deoxynivaneol and other mycotoxins. Canadian J Botany 61: 3080-3087.

Miller JD, Mackenzie S (1999): Secondary metabolites of *Fusarium venenatum* strains with deletions in the Tri5 gene encoding trichodiene synthase. Mycologia

Moreno OM (1977) Acute toxicity study [of longifolene] in rats, rabbits and guinea pigs. Research Institute for Fragrance Materials. Engelwood Cliffs, NJ 07632. USA.

Nandi B, Fries N (1976): Volatile aldehydes, ketones, esters and terpenoids as preservatives against storage fungi in white. J Plant Diseases Protection 83:284-294.

Prelusky, D.B. et al. (1989): Application of the chick embryotoxicity bioassay for the evaluation of mycotoxin toxicity. Microbiologie Aliments Nutrition 7: 57-65.

Rotter RG, Thompson BK, Trenhom HL, Prelusky DB, Hartin KE, Miller JD. (1992): A preliminary examination of potential interactions between deoxynivalenol (DON) and other selected *Fusarium* metabolites in growing pigs. Canadian Journal of Animal Science Vol. 72, pp. 107-116.

Strongman DB, Miller JD, Calhoun L, Findlay JA, Whitney NJ. (1987): The biochemical basis for interference competition among some lignicolous marine fungi. Botanica Marina 30:21-26.

Sweet LI, Meier PG (1997) Lethal and sublethal effects of azulene and longifolene to Microtox (R), *Ceriodaphnia dubia*, *Daphnia Magna* and *Pimephales promelas*. Bulletin of Environmental Contamination and Toxicology 58:268-274.

Wang YZ, Miller JD (1988): Effects of *Fusarium graminearum* metabolites on wheat tissue in relation to *Fusarium* head blight resistance. J Phytopathology 122:118-125.

Table 1 Number of revertant colonies per plate obtained with strains of *Salmonella typhimurium* and *Escherichia coli* following exposure to **Culmorin** in the presence and absence of metabolic activation in the direct plate incorporation assay.

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Test substance ($\mu\text{mol}/\text{plate}$)	Without S9. Mean \pm S.D.				
	TA 1537	TA 98	TA 1535	TA 100	WP2 uvrA
2.500	9 \pm 7	30 \pm 6	15 \pm 1	129 \pm 10	55 \pm 6
1.250	10 \pm 2	37 \pm 6	20 \pm 4	156 \pm 19	64 \pm 10
0.625	8 \pm 3	49 \pm 4	18 \pm 5	158 \pm 34	65 \pm 9
0.313	10 \pm 3	49 \pm 10	20 \pm 10	154 \pm 7	67 \pm 7
0.156	12 \pm 4	28 \pm 4	24 \pm 1	164 \pm 19	60 \pm 6
0.078	15 \pm 4	44 \pm 13	20 \pm 2	152 \pm 20	67 \pm 6
Solvent control	11 \pm 3	44 \pm 8	22 \pm 5	156 \pm 23	65 \pm 7
Ethanol					
9-AA (80 $\mu\text{g}/\text{plate}$)	901 \pm 280				
2-NF (1 $\mu\text{g}/\text{plate}$)		355 \pm 72			
SAz (0,5 $\mu\text{g}/\text{plate}$)			456 \pm 31		
SAz (2 $\mu\text{g}/\text{plate}$)				872 \pm 79	
ENNG (2 $\mu\text{g}/\text{plate}$)					487 \pm 79
	With S9. Mean \pm S.D.				
	TA 1537	TA 98	TA 1535	TA 100	WP2 uvrA
2.500	9 \pm 2	40 \pm 5	14 \pm 2	122 \pm 15	85 \pm 7
1.250	6 \pm 1	41 \pm 3	18 \pm 1	168 \pm 4	90 \pm 10
0.625	8 \pm 3	41 \pm 3	17 \pm 3	166 \pm 13	109 \pm 8
0.313	10 \pm 2	36 \pm 7	12 \pm 3	138 \pm 13	99 \pm 5
0.156	11 \pm 3		16 \pm 2	162 \pm 9	73 \pm 9
0.078	6 \pm 3	37 \pm 5	14 \pm 3	145 \pm 4	78 \pm 4
Solvent control	9 \pm 2	37 \pm 9	15 \pm 3	179 \pm 14	70 \pm 10
Ethanol					
B P (5 $\mu\text{g}/\text{plate}$)	76 \pm 19	238 \pm 5			
2-AA (2 $\mu\text{g}/\text{plate}$)			66 \pm 9		
2-AA (1 $\mu\text{g}/\text{plate}$)				306 \pm 36	
2-AA (20 $\mu\text{g}/\text{plate}$)					623 \pm 51

Abbreviations: 9-AA: 9-Aminoacridine, 2-NF: 2-Nitrofluorene, SAz: Sodium azide,

ENNG: Ethyl-N'-Nitro-N-Nitrosoguanidine, B P: Benzo()pyrene, 2-AA: 2-Aminoanthracene

APPENDIX
B-22

MEMO

Enzyme Business Toxicology

Date : 1999-08-16
File : 1999-07215-01
Ref.: PBjP



Novo Nordisk

Enniatins

ISOLATION AND CHARACTERIZATION

Enniatins (A, A₁₋₂, B, B₁₋₄, D, E, F) are fungal metabolites produced by strains belonging to a variety of species within the genus *Fusarium* (ref.1-3). They are characterised chemically as cyclohexadepsipeptides synthesised by the multifunctional enzyme enniatin synthetase (ref.4). They consist of three D-2-hydroxyisovaleric acid (Hylv) residues linked alternatively to L-amino acids or N-methyl-L-amino acid residues to give an 18-membered cyclic skeleton (ref.3,5)

Types A and B enniatins, which have N-methylisoleucine (NMelle) and/or N-methylvaline (NMeVal) moieties, are produced by fermentation of various strains of fusaria, including *Fusarium acuminatum* (ref.6,7), *F. avenaceum* (ref.5); *F. compactum* (ref.8), *F. lateritium* (ref.9), *F. oxysporum* and *F. scirpi* (ref 10), *F. sambucinum* (ref.11), and *F. tricinctum* (ref.12). Enniatin D, E, and F was described from a *Fusarium* sp. FO-1305 (ref.2). Enniatin C, with three N-methyl-leucine (NMeLeu) moieties, has only been obtained by chemical synthesis (ref. 13).

BIOCHEMICAL AND BIOLOGICAL ACTIVITIES

Enniatins behave as **ionophore compounds** in forming carrier ion complexes with alkali metals with binding preference for the K⁺-ion (ref.14-16). They are known to **uncouple oxidative phosphorylation** in isolated mitochondria, a reaction mediated by induction on an energy-dependent accumulation of potassium ions (ref.17).

Enniatins exhibit various biological activities. Some of these activities have been connected with the ionophoric properties. However, it seems still unclear whether and to what extent these activities are actually derived from the ionophoric properties.

The **antimicrobial activity** against Gram-positive bacteria has been associated partly with the ability to facilitate K⁺-transport (ref.18-20). Enniatins B, B₁, and A₁ were found to exhibit strong antifungal activity against the plant pathogen *Eutypa armeniacea* (ref.21).

Most of the available data on the **insecticidal activity** of enniatins are referred to mixtures of two or more enniatins or to pure cultures of enniatin A and B. A mixture of type A enniatins produced by the entomopathogenic fungus *F. avenaceum* has been shown to be toxic against *Choristoneura fumiferana* Clem. (spruce budworm) at concentrations of 400 ppm and to be toxic at levels of 5 ppm to insect cells in culture (ref.5).

Moderate insecticidal activity against *Calliphora erythrocephala* (blowfly) and *Aedes aegypti* (mosquito) larvae was demonstrated applying mixtures of enniatins from *F. lateritium* (ref.24).

Enniatin A showed an **anthelmintic activity** against *Nippostrongylus brasiliensis*, *Trichonella spiralis* and *Heterakis spumosa* at a concentration of 5 µg/ml, whereas enniatins A₁, B, B₁ had an activity at concentrations between 1 and 100 µg/ml (ref.22).

Enniatin B showed a significant increase in *M.javanica* (a plant parasitic nematode) mortality at 20 ppm.(ref.23).

The mixture B/B₁ has been shown to be **phytotoxic** to wheat seedlings by inhibiting root and leaf development in germinating seeds (ref.25) and the mixture A/B to affect water uptake by cells in tomato shoots, causing the symptoms of toxic wilt (ref.26). Further, it has been demonstrated in an assay on potato tuber tissue, that the production of enniatin contributes to the virulence of the plant pathogen *Fusarium avenaceum* but is not essential for the infection (ref.27). Most recently enniatin was shown to have an inhibitory effect on germination of the plant parasitic weed *Striga hermonthica* of around 50% at 10 mM (ref. 28).

The **cytotoxicity** of Enniatin B was studied by Mereish et al (ref.29). Incubation with 1 and 10 μ M Enniatin B did not release [¹⁴C]adanine nucleotides or LDH in cultured rat hepatocytes.

Tomoda et al. (ref.30) reported cytotoxic activity of enniatins against cultured mouse peritoneal macrophages with the hydrophobic enniatins A, A1, F, and E (CD₅₀: 2.6 to 2.9 μ M) as the most cytotoxic compounds.

MAMMALIAN TOXICITY

Little is known about toxic effects of enniatins on higher animals.

The parenteral, dermal and transdermal toxicity of enniatin produced by *F.oxysporum* have been studied by Wannemacher et al. (ref.31). In a short abstract it is stated: "By the intraperitoneal (ip) route, in the mouse, the LD₅₀ for enniatin was 20.6 mg/kg, with early death in 2-10 minutes and later deaths at 1-4 days. For the subcutaneous (sc) route, the lethal dose was not reached even at 40 mg/kg. We did not observe transdermal lethality in the guinea pig at a dose of 11 mg/ kg and skin irritability was greater than 50 ng/2 μ l spot."

Bosch et al. (ref.32) found no toxic signs when 2 mg of enniatin in 500 μ l of 20% ethanol was administered to 40 g rats. No further details of the enniatin preparation applied or study design were specified.

These data indicate that the toxicity of enniatins to higher animals is low. They are classified as antibiotic metabolites and the mammalian toxicity of enniatins is apparently not an issue of major concern. The presence of natural occurring enniatins in feed and foodstuff is in general not regarded as a risk to the health of humans and livestock.

REFERENCES

1. Visconti, A. et al (1992): Production of Enniatins by *Fusarium acuminatum* and *Fusarium Compactum* in Liquid Culture: Isolation and Characterization of Three New Enniatins, B₂, B₃, and B₄. Journal of Agricultural and Food Chemistry Vol 40 1076-1082.
2. Tomoda et al. (1992): New cyclodepsipeptides, enniatins D, E, and F produced by *Fusarium* sp. FO-1305. Journal of Antibiotics. Vol. 45(8), 1207-1215.
3. Blais, LA et al. (1992). Isolation and characterization of enniatins from *Fusarium avenaceum* DOAM 196490 Canadian Journal of Chemistry Vol 70(5): 1281-1287.
4. von Döhren, H et al (1997): Multifunctional Peptide Synthetases. Chemical Reviews, Vol. 97: 2675-2705
5. Strongman, D.B. et al (1988): Enniatins from *Fusarium avenaceum* isolated from balsam fir foliage and their toxicity to spruce budworm larvae, *Choristoneura fumiferana* (Clem.) Journal of Chemical Ecology, Vol. 14, 753-764.
6. Deol, BS et al (1978): Isolation of cyclodepsipeptides from plant pathogenic fungi. Australian Journal of Chemistry, Vol. 31(6): 1397-1399
7. Drysdale, RB (1984): The production and significance in phytopathology of toxins produced by species of *Fusarium*. In: The Applied Mycology of Fungy. Moss, M.O. and Smith, J.E. eds. Cambridge University Press: Cambridge. pp 95-106
8. Greenhalgh, R et al (1991) Toxicigenic potential of *Fusarium compactum* R8287 and R8293 Journal of Agricultural and Food Chemistry Vol 39 809-812

9. Bishop, GC. and Isley, AH. (1978): Production of Enniatin as a criterion for confirming the identity of *Fusarium lateritium* Australian Journal of Chemistry, Vol. 31: 93-96.
10. Madry, N. et al. (1983): Enniatin production by *Fusarium oxysporum* in chemically defined media. European Journal of Applied Microbiology and Biotechnology Vol. 17: 75-79.
11. Minasyan, AE. et al. (1978): Synthesis of Enniatin B by *Fusarium sambucinum*. Mikrobiologiya Vol.47: 67-71
12. Burmeister, HR. and Plattner, RD. (1987). Enniatin production by *Fusarium tricinctum* and its effect on germinating wheat seed. Phytopathology Vol 77: 1483-1487.
13. Ovchinnikov, YuA. et al. (1964): The enniatin ionophores. Conformation and ion-binding properties. Journal of Peptide and protein Research. Vol 6: 465-498.
14. Benz, R. (1978) Alkali Ion Transport Through Lipid Bi Layer Membranes Mediated by Enniatin A and Enniatin B and Beauvericin Journal of Membrane Biology, Vol. 43(4), 367-394.
15. Shemyakin, MM. et al. (1969): Cyclodepsipeptides as chemicals for studying ionic transport through membranes. Journal of Membrane Biology. Vol.1: 402-430.
16. Pressman, BC. (1976): Biological applications of ionophores. Annual Review of Biochemistry. Vol. 45: 501-530.
17. Akimenko, VK et al. (1981): Interrelationship between Enniatin Synthesis Atp Pool and Cyanide Resistant Respiration in *Fusarium sambucinum*. Microbiology, Vol. 50(2): 141-144
18. Gorneva, G A. et al (1976): Ionophoric Properties and the Mode of Anti Microbial Action of Valinomycin, Enniatins and their Synthetic Analogs. Bioorganicheskaya Khimiya, Vol. 2(9): 1165-1173
19. Tirunarayanan, M.O. and Sirsi, M. (1957): Antibiotics from the genus *Fusarium*. Enniatin B. I. Culture studies and antimicrobial activity. Journal Indian Institute of Science. Vol. 37, 1348-1351.
20. Levy, D. et al. (1995): Alkali cation transport through liposomes by the antimicrobial fusarungine and its constitutive enniatins. Biochemical Pharmacology, Vol. 50(12), 2105-2107.
21. Tsantrizos, YS et al (1993): Novel quinazolinones and enniatins from *Fusarium lateritium* nees. Canadian Journal of Chemistry. Vol. 71(9), 1362-1367.
22. Pleiss, U. et al. (1996) Synthesis of a radiolabeled enniatin cyclodepsipeptide [h-3-methyl]e-1798. Journal of Radiolabeled Compounds and Radiopharmaceuticals, Vol. 38(7), 651-659.
23. Ciancio, A. (1995): Observations on the nematocidal properties of some mycotoxins Fundamental and Applied Nematology, Vol. 18(5), 451-454
24. Grove, J.F. and Pople, M. (1980) The insecticidal activity of beauvericin and the enniatin complex. Mycopathologia Vol. 70 103-105.
25. Burmeister, HR and Plattner, RD (1987): Enniatin production by *Fusarium tricinctum* and its effect on germinating wheat seeds. Phytopathology Vol. 77, 1483-1487.
26. Gaumann, E. et al (1960). Phytotoxic activity of the enniatins. Phytopathology Z., Vol.40, 45-51.
27. Herrmann, M. et al. (1996) Enniatin production by *Fusarium* strains and its effect on potato tuber tissue. Applied and Environmental Microbiology, Vol. 62(2), 393-398.
28. Zonne, MC. (1999) Effect of fungal toxins on germination of *Striga hermonthica* seeds. Weed Research: Vol 39(1): 15-20
29. Mereish, KA. Et al. (1989) Comparative toxicity of cyclic peptides and depsipeptides in cultured rat hepatocytes. Medical Science Research, Vol. 17(20), 869-871.
30. Tomoda et al. (1992). Inhibition of Acyl-CoA: Cholesterol Acyltransferase Activity by Cyclodepsipeptide Antibiotics. Journal of Antibiotics Vol. 45(10), 1626-1632.
31. Wannemacher, RWJr. et al (1988): Parenteral, Dermal, and Transdermal Toxicity of the depsipeptide ionophores, Enniatin and Valinomycin. FASEB (Federation of American Societies for Experimental Biology) Journal , Vol. 2 (5) p. ABSTRACT 6133.
32. Bosch, U. et al (1989). Toxicity and toxin production by *Fusarium* isolates from New Zealand. Mycopathologia Vol 108: 73-79. Kluwer Academic Publishers.

000523

Appendix B-23

To: PBjP, PHva, PBP, AGP, EjBJ, Gram, JeRS, WTY, MWü
From: HeM

5/12/1999
1999-08357-02

Fusarin data in literature.

Summary

Literature information on fusarin C is limited. Chemical knowledge is scarce, but without doubt the main problem (seen from an analytical point of view) is lability. Nothing is known about inducing conditions for *F. venenatum*, but these conditions are known for a number of other *Fusarias* and especially *F. moniliforme*. Information is, however, confusing, and no general rule regarding optimal conditions for fusarin C production can be formulated. The biosynthetic pathway is not known in any detail, and no genes are known.

Background

Fusarin may be present in *F. venenatum*. Therefore it is interesting to know as much as possible about this compound. For the work in Microbial Metabolism it is especially important to focus on chemical and physical data and data regarding the induction of the biosynthesis of the compound. The literature on the topic, which is limited due to relatively little attention from academia, is summarised here. PBjP makes a review of toxicological aspects. In order to give an indication of toxicity it was found in one study (14) that 1 mM (400 ppm) fusarin C is necessary to cause cell death of rat hepatocytes..

Chemical data

Fusarins are a family of compounds (polyketides) including isomers of fusarin C. In several studies 3 stereo-isomers of fusarin C were identified by NMR (3, 7). The isomers are separated by C18-HPLC and are formed after irradiation with light at 366 nm for 5 min at room temperature. Spectroscopic data on fusarin C including both ¹H and ¹³C NMR spectra are presented in reference 7 and in ref. 8 for the other fusarins. The molecular ion of fusarin C is 431.2 and the absorbency maximum in methanol is 358 nm (also shown in 12 and 16). Fusarin C has also been analysed by TLC and by gas chromatography as the trimethylsilyl ester derivative (13).

Jackson et al (18) have described a quantitative HPLC method for fusarin C measurement. They also observe rapid isomerization of fusarin C even when the standard is frozen. Thus they postulate that quantification must include several peaks. They observe that even in the dark under frozen conditions more than 20% fusarin is lost in the first 10 days of storage. After that degradation is less fast. They recommend the use of an internal standard of phenothiazine.

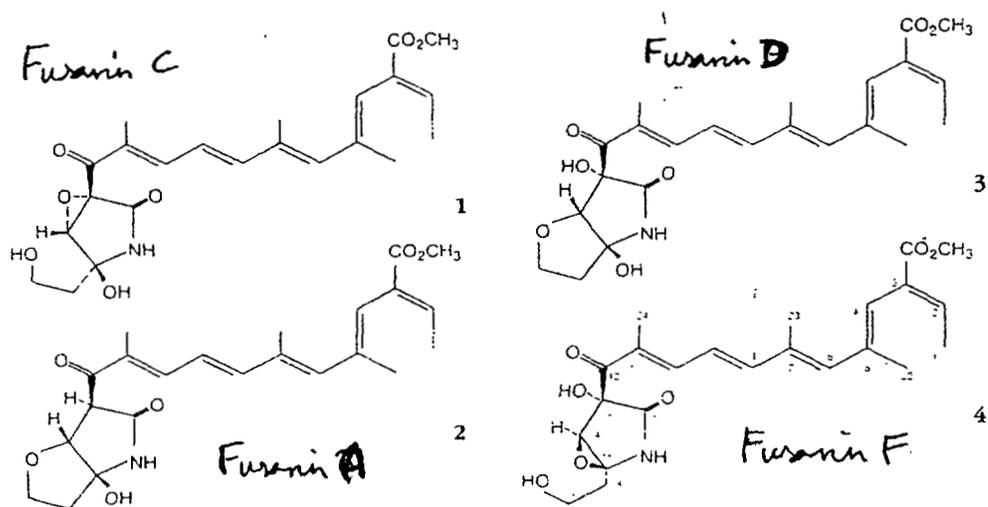
Fusarin C, which is the most thoroughly studied compound of the family is labile to heat and light (especially long wave UV, 10). "Prolonged" exposure to light (5 min.) under some conditions leads to loss of the chromophores. At the same time the mutagenicity of the compound disappears.

Studies of the stability of fusarin C as a function of pH and temperature (15, 16) shows that the compound is relatively stable at pH-values below 6.5 even at higher temperatures. However, under alkaline conditions the compound degrades fast.

Fusarin C is very sensitive to glutathione (reducing conditions in general? (16)) which degrades it to fusarin A and a compound without the 2-pyrrolidone, especially at pH-values above 7 (11). This is a very efficient means to remove any mutagenic activity, and this can potentially be used in production. Another likely degradation is demethylation of the ester group occurring in vivo (11). This compound is more water-soluble than fusarin C. Fusarin C is often kept in chloroform to be used as standard (e.g. 11).

Fusarin C is normally extracted and purified in ethyl acetate or chloroform. Alternatively it has been purified on silica-gel columns (3, 12, 18). Fusarin F seems to be especially labile during recovery (8).

000524



¹PRC Publication No. 1339

Figure 1: from ref 8

The other fusarins (see figure, A, D, E, and F) except F are non-mutagenic. Their relative molecular weights are 415, 431, ?, and 431, respectively (7). Only fusarin C and probably F have been found to be mutagenic most likely due to the presence of the epoxy-bonds. Fusarin B has never been isolated (8).

Lu and Jeffrey (16) found a fusarin X which is the 1-hydroxy-analog of fusarin C in some strains of *F. moniliforme*. Its properties with respect to temperature, pH, and reducing environment sensitivity corresponds to fusarin C. Reduction by glutathione and degradation by heat seems to lead to the same product with a characteristic UV spectrum with an absorption maximum at 320 nm. Fusarin X is mutagenic.

During HPLC on a C18-column Savard and Miller got the following elution order: F, C, A. (8). Fusarin F readily isomerises into fusarin C (8).

Fusarin:	C	A	D	E	F
Molecular weight (dalton)	431	415	431	?	431
Absorption maximum (nm)	353-358				370 (shoulder at 293 nm)
Toxicity	mutagenic	nonmutagenic	nonmutagenic	nonmutagenic	mutagenic

Due to problems with stability the reported concentrations are relative values.

Biosynthetic pathway

Fusarins consist of a polyene with a substituted 2-pyrrolidone moiety (see figure). Studies indicate that the polyene is formed from acetyl-CoA and 6 malonyl-CoA units and oxaloacetate giving the polyketide, which is coupled to the 2-pyrrolidone of unknown origin (5). Savard and Miller believe that fusarin F is a precursor of fusarin C (8). Fusarin C is usually dominant in *F. moniliforme* with slightly lower levels of fusarin F(9).

Expression of fusarins

All experiments below are done in shake flasks with this methods inherent weakness in the form of drift in pH etc. Especially with a labile compound as fusarin C decreasing levels may be seen in spite of continuing production if the conditions with respect to stability deteriorates. This can thus mistakenly be taken as no production.

000525

Fusarins are according to literature found in *Fusarium graminearum*, *F. moniliforme*, *F. avenaceum*, *F. culmorum*, *F. poae*, *F. sambucinum*, and *F. sporotrichioides* (1) while it has not been found in *F. solani*, *F. glutinans*, *F. semitectum*, *F. equiseti*, and *F. acuminatum*. Inducing conditions may not be similar and this may explain part of the "confusion" regarding this topic. The compounds have also been found in *Gibberella fujikuroi* (12), where a new cis-isomer of fusarin C was also observed. Production of fusarins is often recognized by a yellowish colour in the mycelium or the culture medium.

Aeration, temperature, and pH are crucial for the induction of fusarin C biosynthesis.

Farber and Sanders (1) found for a number of strains that glucose/yeast extract/peptone (GYEP) does lead to fusarin C production while Czapek-Dox and MYRO media do not. No or slow shaking is optimal indicating that a low oxygen concentration is preferred for *F. graminearum* which is one of the most efficient producers. Inoculum size is also very important. 28°C and an initial pH below 7 (5.9 to 7.5 tested) are optimal for this strain. The lower optimal pH limit was not found in the study. Glucose has a significant negative effect on fusarin C production. Levels up to 25 ppm are observed.

Conditions for fusarin C production in *F. moniliforme* are different from what is described above (2, 6). A high sugar concentration (preferably glucose and especially sucrose) in MYRO medium, a pH around 4, and aeration gives the largest production (above 60 ppm with an extreme of 450 ppm). Temperature is optimal between 25 and 35°C. The time course of the mycotoxin production indicates primarily production under growth. Before the initiation of the production pH had dropped to 3. Both ammonia and glucose were abundant during the production phase. Thus it was concluded that neither nitrogen nor glucose limitation is needed for stimulation of fusarin C biosynthesis in *F. moniliforme*. Aeration should not be too vigorous. That the production is largest during exponential growth was confirmed in a later study in a defined medium with 8% glucose initially (3). This concentration does, however, seem to limit toxin production for a while (2 days). Jackson et al also found that glucose surplus is necessary and further observed that zinc limitation leads to fusarin C production which is paralleled with increased lipid production (4, 17, 19). In the absence of zinc, manganese induced fusarin production while cultures supplied with iron, cobalt, zinc and manganese produce the smallest amount of the toxin. Zinc limitation also stopped the ethanol production normally seen with excess glucose. A zinc surplus (3 ppm) seems to limit fusarin C biosynthesis, especially when carbon source is low. Urea, nitrate, and ammonia seems to give larger fusarin concentrations than when nitrogen source is supplied as amino acids or protein but it may be due to the inherent addition of extra zinc in the complex sources (19). An explanation for the zinc effect given by Jackson et al is that nitrogen starvation probably occurs in the absence of zinc since the ammonia assimilation is seriously affected under these conditions. Complex nitrogen sources are not as affected giving a better growth with less fusarin production.

Mutants with a higher fusarin production have been isolated in *G. fujikuroi* (12)

References.

1. Farber, MJ, and Sanders, GW (86) J. Agric. Food Chem. 34, 963
2. Farber, MJ, and Sanders, GW (86) Appl. Envir. Microbiol. 51, 381
3. Cantalejo, MJ et al (97) J. Food Protection 60, 433
4. Jackson, MA, and Lanse, AC (93) FEMS Microbiol. Lett. 108, 69
5. Steyn, P, and Vlegaar, R (85) J. Chem. Soc., Chem. Commun. , 1189
6. Tseng, T-C, and Tseng, T-I (92) Bot. Bull. Acad. Sin. 33, 179
7. Gelderblom, WCA et al (84) J. Chem. Soc., Chem. Commun. , 122
8. Savard, ME, and Miller, JD (92) J. Nat. Prod. 55, 64
9. Miller, JD, et al (93) Mycologia 85, 385
10. Gelderblom, WCA, et al (83) Toxicon 21, 467
11. Gelderblom, WCA, et al (88) Mutation Res. 199, 207
12. Barrero, AF et al (91) Phytochemistry 30, 2259
13. Tseng, T-C, and Tseng, T-I (90) Bot. Bull. Acad. Sin. 31, 169
14. Norred, WP et al (91) Mycopathologia 115, 37
15. Zhu, B and Jeffrey, AM (92) Nutrition and Cancer 18, 53
16. Lu, F-X, and Jeffrey, AM (93) Chem. Res. Toxicol. 6, 91
17. Jackson, MA et al (89) Appl. Envir. Microbiol. 55, 649
18. Jackson, MA et al (90) J. Agric. Chem. 38, 1511
19. Jackson, MA and Freer, SN (91) FEMS Microbiol. Lett. 82, 323

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Memo

Enzyme Business Toxicology

Date : 30th November 1999

File No.: 1999-09295-02

Ref.: PBjP

To : HeM, AGP, PBP, PHva.

Copy : ABe

From : PBjP

**Re : Toxicity of the Secondary Metabolite Fusarin C produced by *Fusarium* spp.
A literature survey.**

Introduction

The production, chemical and physical properties, and toxicology of fusarin C has been comprehensively reviewed by Farber and Scott (1989) and in an IARC (International Agency for Research on Cancer) monograph from WHO (1993). We haven't found any significant information in the more recent literature which may contribute to assess human health risks from exposure to fusarin C.

The conclusion on the toxicology of Fusarin C is summarised below. For more details and references see reviews above.

General toxicity to animals.

The acute and chronic toxicity of fusarin C has not yet been established, possible because of lack of sufficient quantities of pure material to carry out toxicity studies.

However, in the carcinogenicity studies by Gelderblom et al. (1986), an oral dosage of 100 mg fusarin C/kg bw followed by phenobarbital treatment was lethal to all of five female Wistar rats and two of five male BD IX rats. All rats survived a dosage of 50 mg fusarin C/kg bw. This result indicate a modest acute mammalian toxicity.

Norred et al. (1991) found a positive correlation between the cytotoxicity of organic extracts from a strain of *F.moniliforme* and the content of fusarin C. 10^{-4} M of purified fusarin C inhibited (^3H) valine incorporation into proteins of primary rat hepatocytes and cell death occurred at 10^{-3} M.

The instability of Fusarin C to heat is well established (Zhu and Jeffrey 1992, Gelderblom et al. 1983, Scott et al. 1986). It is most easily destroyed by cooking especially at slightly basic pH values and even at room temperature it is unstable. However, when fusarin C is subjected to the conditions found in the stomach (30 minutes at 37°C, pH from 1.0 to 7.0) fusarin C was rather stable (less than 15% decomposition) (Zhu and Jeffrey, 1992)

000528

Mutagenicity

Fusarin C is mutagenic *in vitro*.

Mutagenicity at the gene level has been demonstrated several times in *Salmonella* bacteria (Wiebe and Bjeldanes 1981, Gelderblom et al. 1983, Gelderblom and Snyman 1991). In mammalian cells cytogenetic effects such as induced sister chromatid exchanges, chromosomal aberrations, and micronuclei have been demonstrated in Chinese hamster V79 lung cells (Cheng et al. 1985).

The C₁₃-C₁₄ epoxide moiety seem to be essential in order to express mutagenic activity, although activation by an exogenous metabolic system is further required. Deactivation of the mutagenic metabolite formed seemed to occur through chemical binding to thiol groups and by enzymatic conjugation mediated by a cytosolic glutathione-S-transferase (Gelderblom et al. 1984).

Two metabolites, fusarin Z and fusarin X, were isolated and identified after metabolic conversion of fusarin C by induced rat liver microsomal mixtures (Zhu and Jeffrey 1993). Both metabolites resulted from hydroxylation at the 1-position of fusarin C. They were 500 and 60 times more mutagenic than fusarin C in the Ames test, respectively, and they could account for much of the mutagenic activity of activated fusarin C.

Lu et al. (1988) did not observe formation of DNA adducts in *Salmonella typhimurium* or in isolated calf thymus DNA *in vitro* by ³²P-postlabelling. However, DNA replication was induced in polyoma transformed rat embryo fibroblasts without an exogenous metabolic system and gene mutations of the base-pair substitution and frame-shift mutation type as well as strand breaks in *Salmonella typhimurium*, but only in the presence of metabolic activation.

Norred et al. (1992) investigated the ability of fusarin C (1.0-100 µM) to induce unscheduled DNA synthesis (UDS) in primary hepatocytes. The results were inconclusive since only a marginal effect was obtained. It was argued that inactivation of fusarin C by conjunction with glutathione is more likely to occur in the UDS-assay than in other *in vitro* assays applying rat liver homogenates (S9) as an exogenous metabolic activation system.

Lu et al. (1991) have reported the *in vitro* transformation of rat esophageal epithelial cells by fusarin C (in Chinese). According to the English abstract the transforming activity was established by cloning of transformed cells, increased chromosome number, enhanced expression of oncogenes, and development of squamous cell carcinomas after inoculating the cells into nude mice.

No *in vivo* mutagenicity test has been reported with Fusarin C.

Carcinogenicity

In consideration of the strong *in-vitro* mutagenicity of Fusarin C and the fact that it occurs naturally in corn in many parts of the world, the evaluation of carcinogenicity of this metabolite is important.

Only one report describes induction of carcinogenicity in mammals (Li et al 1992). This paper is written in Chinese, and a translation has not been available to the author. The following is cited from the IARC-monograph (1993):

"A group of 29 female DBA mice, 8-10 weeks of age, were given 0.5 mg fusarin C [purity unspecified] by gavage twice a week; when toxic effects became apparent, the dose was decreased to 0.05 mg twice a week. A control group of 20 mice was available. Animals were evaluated for development of forestomach and esophageal tumours and were observed to a maximum of 655 days after initiation of dosing. Dysplasia in the forestomach and esophagus was observed 2/28 treated animals, papillomas of the forestomach and esophagus in 3/28 and carcinomas of the forestomach and esophagus in 3/28. There was no evidence of such lesions in the control group."

000529

"A group of 20 female Wistar rats, weighting 80-120 g, were given 2 mg fusarin C [purity unspecified] by gavage twice a week; as body weight increased, the dose of fusarin C was increased to 3 mg twice a week. A control group of 25 rats was available. Animals were evaluated for development of forestomach and eosophageal tumours and were observed to a maximum of 742-814 days. Dysplasia in the forestomach and eosophagus was observed 1/20 treated animals, papillomas of the forestomach and eosophagus in 5/20 and carcinomas of the forestomach and eosophagus in 5/20. There was no evidence of such lesions in the control group."

With reservation to the purity of the test material, this study represents the only experimental evidence for a carcinogenic effect of fusarin C in animals. Zhu and Jeffrey (1992) mention that the fusarin C, used by Li et al (1992), was 20% and 86% pure for the rat and mice experiments, respectively (source of information unmentioned). The extracts were dissolved in ethanol before dilution in water. The final concentration of ethanol was not given, and it is unclear whether the controls were treated with the solvent.

Other studies have failed to demonstrate carcinogenic activity of fusarin C.

In an investigation by Jasciewicz et al. (1987) rats were fed diets added culture material from two different strains of *Fusarium moniliforme* containing 0.5 mg and 18.2 mg fusarin C respectively per kg diet. After prolonged exposure, all rats receiving culture material with low fusarin C content developed neoplastic lesions in the liver and increased incidences of forestomach papillomas and carcinomas were observed. (It was later found that the strain of *Fusarium moniliforme* producing the culture material with low fusarin C content is a high producer of fumonisin B₁, a now well known carcinogen.). In rats fed diets with high fusarin C content the incidence of neither liver nor forestomach tumours was significantly increased. These data indicate, that fusarin C is non-carcinogenic.

Gelderblom et al. (1986) investigated the cancer initiating potential of fusarin C on mouse skin and in a rat liver initiation/promotion model for carcinogenesis.

Mice were treated with a single application of 220 and 500 µg fusarin C followed one week later by application of the promotor 12-O-tetradecanoylphorbol 13-acetate (TPA). One out of ten mice that received 220 µg fusarin C followed by TPA had two skin papillomas, but there were no skin tumour in mice that received 500 µg fusarin C. No papillomas was seen in the control group painted with only TPA.

Two different strains of rats were fed synthetic diet and subjected to a two-third hepatectomy. One day after surgery rats were given a single dose of 0,50 or 100 mg/kg bw of fusarin C. Carcinogenesis was measured by the presence of altered hepatocytes, as revealed by γ-glutamyltranspeptidase positive staining. Fusarin C did not initiate carcinogenesis and it was not believed to be hepatocarcinogenic in rats because of its rapid conjunction with glutathione and excretion (Gelderblom et al. , 1988)

Unfortunately, the purity of the fusarin C preparation applied in the studies reported by Jasciewicz et al. (1987) and Gelderblom et al. (1986) was unspecified.

Immunological effects

An immunosuppressive activity of fusarin C have been demonstrated at lower concentrations than those required for mutagenesis.

Chen and Zhang (1987) showed that the presence of 2.5 µg/ml fusarin C in lymphocyte cultures significantly inhibited the secondary lympho-proliferative response. Fusarin C not only affect the proliferation of T-lymphocytes but also inhibit the accessory cell function of spleen adherent cells.

Treatment of macrophages *in vitro* with fusarin C (6 µg/ml) inhibited their activation by macrophage activating factor and muramyl dipeptide and strongly inhibited the cytotoxic function of activated macrophages. These effects were dose-dependent and reversible and not due to a general cytotoxic activity (Dong and Zhang 1987).

The authors suggest, that the possible role of fusarin C in a potential carcinogenesis may be two fold, i.e., to induce cell mutation and malign transformation and at the same time to inhibit the immune-surveillance mediated by activated macrophages.

000530

Conclusion

Based on the limited toxicological data that exists it is presumable that the acute toxicity of Fusarin C to humans and animals is low.

Fusarin C is a strong mutagen *in vitro*, although requiring metabolic activation. The mutagenic potential *in vivo* is unknown.

Immunosuppressive activity has been demonstrated at lower concentrations than those required for mutagenesis *in vitro*.

The carcinogenic potential of Fusarin C is controversial. The overall evaluation by the IARC working group is:

There is limited evidence in experimental animals for the carcinogenicity of fusarin C.

This standard conclusion is based on the lack of adequate studies on the carcinogenicity of fusarin C. The evidence of carcinogenicity is restricted to a single experiment in which the extracts used as test substance were of such low purity that it is difficult with certainty to ascribe the tumours specifically to fusarin C.

Finally, it is important to establish, that animal carcinogenicity studies cannot be used as definitive evidence with respect to human. There may be species differences in metabolism, response to fusarin C or the requirement for specific tumour promoters.

References

- Chen, L. and Y. Zhang (1987): Suppression of the *in vitro* lympho-proliferative response to syngeneic LS178Y tumor cells by Fusarin C in mice. *Journal of Experimental and Clinical Cancer Research*, Vol. 6(1), pp 25-29.
- Cheng, S.J et al. (1985): A mutagenic metabolite produced by *Fusarium moniliforme* isolated from Linxian country, China. *Carcinogenesis*, Vol. 6(6), pp. 903-905.
- Dong, Z. and Y. Zhang (1987). Inhibitory effect of a mycotoxin, fusarin C, on macrophage activation and macrophage mediated cytotoxicity to tumor cells in mice. *Journal of Experimental and Clinical Cancer Research*, Vol. 6(1), pp. 31-38.
- Farber, J.M and Scott, P.M. (1989): Fusarin C In Chelkowsky , J., ed., *Fusarium Mycotoxins, Taxonomy and Pathogenicity*, Amsterdam, Elsevier, pp. 41-55.
- Gelderblom, W.C.A et al (1983): A mutagen produced by *Fusarium moniliforme*. *Toxicol*; Vol.21, pp.467-473.
- Gelderblom, W.C.A et al (1984): Metabolic activation and deactivation of fusarin C, a mutagen produced by *Fusarium moniliforme*. *Biochemical Pharmacology*. Vol 33(10), pp.1601-1603.
- Gelderblom, W.C.A. et al. (1986): Investigations on the carcinogenicity of Fusarin C a mutagenic metabolite of *Fusarium moniliforme*. *Carcinogenesis*, Vol. 7(11), pp. 1899-1901.
- Gelderblom, W.C.A. et al. (1988): The chemical and enzymatic interaction of glutathione with the fungal metabolite, fusarin C. *Mutation Research*, Vol.199, pp. 207-214.
- Gelderblom, W.C.A. and Snyman, S.D (1991): Mutagenicity of potential carcinogenic mycotoxins produced by *Fusarium moniliforme* *Mycotoxin Research*, Vol.7, pp. 46-52.
- IARC (1993): Toxins derived from *Fusarium moniliforme*: Fumonisin B1 and B2 and Fusarin C *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*; Vol.56, pp. 445-466. (Last updated 08/21/1997).
- Jasciewicz, K. et al (1987): Carcinogenicity of *Fusarium moniliforme* Culture Material in Rats *Journal of the National Cancer Institute*; Vol.78, pp. 3421-325
- Li, M. et al (1992): Carcinogenicity of Fusarin C isolated from *Fusarium moniliforme*". *Chinese Journal of Cancer Research* Vol 2; pp. 1-5
- Lu, F-X. et al (1991): *In vitro* transformation of rat esophageal epithelial cells by fusarin C. *Science in China Series B Chemistry Life Sciences and Earth Sciences*. Vo. 34(12), pp. 1469-1477. (in Chinese)
- Lu, S -J. et al. (1988): *Fusarium moniliforme* metabolites. genotoxicity of culture extracts *Carcinogenesis*, Vol. 9(9), pp. 1523-1527.

000531

Norred, W.P. et al. (1991): Differential cytotoxicity and mycotoxin content among isolates of *Fusarium moniliforme*. *Mycopathologia*; Vol.115(1), pp. 37-43.

Norred, W.P. et al. (1992): Effects of selected secondary metabolites of *Fusarium moniliforme* on unscheduled synthesis of DNA by rat primary hepatocytes. *Food and Chemical Toxicology*; Vol.30(3), pp. 233-237.

Scott, P.M. et al (1986): Analysis of toxins of *Fusarium moniliforme*. In P.S.Steyn and R Vleggaar (Eds.): *Mycotoxins and Phycotoxins*, Elsevier Science Publications, Amsterdam.

Wiebe, L.A. and Bjeldanes, L.F. (1981): A mutagen from *Fusarium moniliforme* grown on corn. *Journal of Food Science*. Vol. 46, pp. 1424-1426.

Zhu, B. and Jeffrey, A.M. (1992): Stability of Fusarin C: Effects of the normal Cooking Procedure Used in China and pH. *Nutrition and Cancer*. Vol.18(1); pp. 53-58.

Zhu, B and Jeffrey, A.M. (1993): Fusarin C Isolation and identification of two microsomal metabolites. *Chemical Research in Toxicology* Vol.6(1); pp. 97-101.

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APPENDIX C - Enzyme Identity and Characterization Information

- * 1. FDA letter to Novo Nordisk, regarding January 1997 meeting on xylanase enzyme preparation produced by *Aspergillus oryzae* expressing the gene encoding a xylanase from *Thermomyces lanuginosus*.
- 2. International Union of Biochemistry and Molecular Biology. Nomenclature Committee. Enzyme Nomenclature - Recommendations (1992) of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology. Academic Press, Inc. 1992.
- * 3. Product Sheet for Pentopan Mono BG
- * 4. Product Sheet for Novozym 899



Novo Nordisk

APPENDIX
C-1



Dr. Scott Shore
Box 576
Novo Nordisk BioChem North America, Inc.
State Road 1003
Franklinton, NC 27525-0576

Dear Dr. Shore:

This letter is in regards to Novo Nordisk's discussion with representatives of the Food and Drug Administration (FDA) about the food use of xylanase enzyme derived from fermentation by a genetically modified *Aspergillus oryzae* microorganism.

Your firm submitted a summary of its safety assessment of the xylanase enzyme preparation and met with representatives of the Center for Food Safety and Applied Nutrition (CFSAN) on January 16, 1997, to discuss this safety assessment. As noted in our meeting of January 16, 1997, xylanases, which are types of pentosanases, are the most functional enzymes in the hemicellulase enzyme preparations, which are well known, in the baking industry.

The summary document provides the rationale for Novo Nordisk's determination that the xylanase enzyme preparation produced from the *Aspergillus oryzae* microorganism that is expressing a xylanase gene from a *Thermomyces lanuginosus* microorganism is generally recognized as safe (GRAS). The document includes information regarding the description of production microorganism, including a review of the safe food use of *Aspergillus oryzae*, the manufacturing process, the enzyme identity, the mode of action of the xylanase enzyme preparation in bread making. The summary document also included summaries of toxicological studies conducted on the enzyme preparation including, a gene mutation assay, a chromosome aberration assay (in vitro cytogenetics with human lymphocytes) and a subchronic toxicity test in rats (13 weeks).

As you are aware, the Federal Food, Drug and Cosmetic Act (FFDCA) does not restrict the determination of the GRAS status, of a substance to FDA alone. Rather, it explicitly recognizes that a consensus among scientific experts regarding the safety of a food substance may form the basis for whether such a substance is GRAS. Thus manufacturers may market a product based on their own determination that a substance is GRAS, provided that the determination is accurate. Such independent determinations are not accompanied by any explicit review, affirmation, or authorization by FDA. FDA concurrence on the GRAS status of a substance is currently achieved through the GRAS affirmation petition process, 21 CFR 170.35.

000536

We appreciate your sharing with us the information on which you have relied to make your determination of GRAS status for xylanase enzyme preparation produced by *Aspergillus oryzae* expressing the gene encoding xylanase from *Thermomyces lanuginosus*. As you are aware, it is Novo Nordisk's responsibility to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

Sincerely yours,

George Pauli, Ph.D.
Director
Division of Product Policy, HFS-205
Office of Premarket Approval
Center for Food Safety
and Applied Nutrition

000536.001

APPENDIX
C-3

Enzyme Business

Novozym 899

- Draft Description** Novozym 899 is a purified endo 1.4-beta-xylanase (pentosanase) from *Thermomyces lanuginosus* produced by submerged fermentation of a genetically modified *Fusarium venenatum* microorganism.
- Application** Novozym 899 improves the elasticity of the gluten network in bread dough by acting on both the soluble and insoluble pentosans in flour. The results are doughs with improved handling and stability and loaves with improved crumb structure and larger volume. Novozym 899 can be used as an alternative to or in combination with dough conditioning emulsifiers in all types of bread. It is inactivated during the baking process.
- Activity** The product is standardized in Fungal Xylanase Units (Wheat). The analytical method is available on request.
- Dosage** Due to its purity, the dosage of Novozym 899 in terms of milligrams of enzyme protein is significantly lower than traditional pentosanases. The recommended dosage is 1-8 grams/cwt. flour (2-16 grams/100 kilos; 50-400 FXU(W)/kg flour). The optimum dosage should be determined through baking trials. Overdosing can result in dough stickiness due to a loss of water retention.
NOTE *Novozym 899 is virtually free of alpha-amylase activity. For optimum results, fungal alpha amylase should be added with the xylanase in an amount equivalent to 25,000-45,000 SKB/cwt. flour (15-25 FAU/kg flour or 0,6-1 g Fungamyl 2500 BG per 100 kg flour.)*
- Product Type** Novozym 899 is standardized using a special wheat flour with a narrow particle size distribution. The product is a light brown, free-flowing, non-dusting, agglomerated powder with an average particle size of 150 microns and a size fraction within 50-212 microns.
- Product Characteristics** Novozym 899 can be used in the pH range of 4-6 and at temperatures up to 75°C (165°F).
Novozym 899 is inactivated in the baking process.
- Solubility** The active components of Novozym 899 are readily soluble in water at concentrations that occur in normal usage. However, water solutions will be turbid because of the wheat flour used for standardization of the enzyme.
- Handling Precautions** Novozym 899 can easily be mixed with flour or starch. Preparing a pre-mix 1:10 can facilitate its use. Recommendations are given in "How to Mix Novo Nordisk Granulated Enzymes" (B 425), which is available on request.

000538



Novo Nordisk

Safety

Enzymes are proteins and inhalation of dust or aerosols may induce sensitization and may cause allergic reactions in sensitized individuals. Some enzymes may irritate the skin, eyes and mucous membranes upon prolonged contact.

The product is developed to resist light mechanical effects. However, excessive mechanical wear and tear or crushing may create dust. All spills, even small spills, should be gently shovelled into plastic-lined containers. Use respiratory protection. Small spills and remains of large spills should be removed by vacuuming or flushing with water (avoid splashing). Vacuum cleaners and central vacuum systems should be equipped with HEPA filters.

A Material Safety Data Sheet and separate material describing how to handle the product safely are available upon request.

Storage

Enzymes gradually lose activity over time depending on storage temperature and humidity. Cool and dry conditions are recommended.

When stored in closed containers at 25°C, Novozym 899 will maintain its declared activity for 3 months.

When stored at 5°C Novozym 899 will maintain its declared activity for 12 months.

Extended storage and/or adverse conditions, including higher temperature or high humidity, may lead to higher dosage requirement.

Enzyme Business

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Laws, regulations and third party rights may prevent customers from importing, processing, applying and/or reselling certain products in a given manner. It is the responsibility of the customers that their specific use of products from Novo Nordisk does not infringe relevant laws and regulations and, furthermore, does not infringe patents or other third party rights

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000539

Appendix
C-4



Pentopan[®] Mono BG

Description Pentopan Mono BG is a purified endo 1.4-beta-xylanase (pentosanase) from *Thermomyces lanuginosus* produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism.

Application Pentopan Mono BG improves the elasticity of the gluten network in bread dough by acting on both the soluble and insoluble pentosans in flour. The results are doughs with improved handling and stability and loaves with improved crumb structure and larger volume. Pentopan Mono can be used as an alternative to or in combination with dough conditioning emulsifiers in all types of bread. It is inactivated during the baking process.
An application sheet (B 993) is available on request.

Activity Pentopan Mono BG is available as:

Pentopan Mono BG..... 2500 FXU(W)/g
Fungal Xylanase Units (Wheat).

The product is standardized with wheat flour by "Documented Addition" in a strictly ISO-controlled process. "Documented Addition" is required as the use of wheat flour for the standardization of the activity can lead to biased assay results due to its similarity to the substrate used for the assay.
A description of the analytical method and a detailed explanation of "Documented Addition" are available on request.

Dosage Due to its purity, the dosage of Pentopan Mono in terms of milligrams of enzyme protein is significantly lower than traditional pentosanases. The recommended dosage is 1-6 grams/cwt. flour (2-12 grams/100 kilos; 50-300 FXU(W)/kg flour). The optimum dosage should be determined through baking trials. Overdosing can result in dough stickiness due to a loss of water retention.
NOTE: Pentopan Mono is virtually free of alpha-amylase activity. For optimum results, fungal alpha-amylase should be added with the xylanase in an amount equivalent to 25,000-45,000 SKB/cwt. flour (5-25 FAU/kg flour or 0,2-1 g Fungamyl[®] 2500 BG per 100 kg flour).

Product Type Pentopan Mono BG is standardized using a special wheat flour with a narrow particle size distribution. The product is a light brown, free-flowing, non-dusting, agglomerated powder with an average particle size of 150 microns and a size fraction within 50-212 microns.

000541



Specification	Pentopan Mono BG complies with JECFA and FCC recommended purity specifications for food-grade enzymes.
Product Characteristics	Pentopan Mono BG can be used in the pH range of 4-6 and at temperatures up to around 75°C (165°F). Pentopan Mono BG is inactivated in the baking process.
Packing	Pentopan Mono BG is available in 60-litre fibre drums with a net weight of 25 kg.
Solubility	The active components of Pentopan Mono BG are readily soluble in water at concentrations that occur in normal usage. However, water solutions will be turbid because of the wheat flour used for standardization of the enzyme.
Handling Precautions	Pentopan Mono BG can easily be mixed with flour or starch. Preparing a pre-mix 1:10 can facilitate its use. Recommendations are given in "How to Mix Novo Nordisk Granulated Enzymes" (B 425), which is available on request.
Safety	Enzymes are proteins and inhalation of dust or aerosols may induce sensitization and may cause allergic reactions in sensitized individuals. Some enzymes may irritate the skin, eyes and mucous membranes upon prolonged contact. The product is developed to resist light mechanical effects. However, excessive mechanical wear and tear or crushing may create dust. All spills, even small spills, should be gently shovelled into plastic-lined containers. Use respiratory protection. Small spills and remains of large spills should be removed by vacuuming or flushing with water (avoid splashing). Vacuum cleaners and central vacuum systems should be equipped with HEPA filters. A Material Safety Data Sheet and separate material describing how to handle the product safely are available on request.
Storage	Enzymes gradually lose activity over time depending on storage temperature and humidity. Cool and dry conditions are recommended. When stored in closed containers at 25°C, Pentopan Mono BG will maintain its declared activity for 3 months. When stored at 5°C Pentopan Mono BG will maintain its declared activity for 12 months. Extended storage and/or adverse conditions, including higher temperature or high humidity, may lead to higher dosage requirement.

Enzyme Business

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Laws, regulations and third party rights may prevent customers from importing, processing, applying and/or reselling certain products in a given manner. It is the responsibility of the customers that their specific use of products from Novo Nordisk does not infringe relevant laws and regulations and, furthermore, does not infringe patents or other third party rights.

The contents of this document are subject to change without further notice.



Appendix

D

000543

APPENDIX D - Manufacturing Process References

1. Aunstrup et al: Production of Microbial Enzymes, Microbial Technology, Academic Press, 1979, 2nd Edition, Vol. 1, 281-309
2. Aunstrup, K.. *Production, Isolation, and Economics of Extracellular Enzymes* in Applied Biochemistry and Bioengineering, Volume 2, Enzyme Technology, Eds. Wingard, L.B., Katchalski-Katzir, E. And Goldstein, L, pp. 28-68, 1979.
3. Enzyme Applications in Encyclopedia of Chemical Technology, 4th ed., Ed. Kroschwitz, J.I., Volume 9, pp. 567-620, 1994.
4. Environmental Protection Agency. Pesticide Tolerance Petition: Notice of Filing. Fed. Regist. 61:58684-58688, 1996.
5. Environmental Protection Agency. Phosphinothricin Acetyltransferase and the Genetic Material Necessary for Its Production in All Plants; Exemption From the Requirement of a Tolerance On All Raw Agricultural Commodities. Fed. Regist. 62:17717-17720, 1997.
6. Organisation for Economic Cooperation and Development, Series on Harmonization of Regulatory Oversight in Biotechnology No. 11, *Consensus document on general information concerning the genes and their enzymes that confer tolerance to phosphinothricin herbicide*, 1999.



Novo Nordisk

000544

APPENDIX

E

000545

APPENDIX E - Safety Reference

- * 1. Pedersen, P. B. and Broadmeadow, A., 2000. Safety evaluation of the *Thermomyces lanuginosus* xylanase expressed by *Fusarium venenatum*, intended for use in food. Food Additives & Contaminants. In Press.



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APPENDIX

E-1

000547

Pages 000548 - 000556 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

SUBMISSION END

000557

Reference List for Industry Submission, GRN 000054

<i>Pages</i>	<i>Author</i>	<i>Title</i>	<i>Publish Date</i>	<i>Publisher</i>	<i>BIB_Info</i>
000495 - 000502	Miller, J. David; MacKenzie, Sabrena	Secondary metabolites of Fusarium venenatum strains with deletions in the Tri5 gene encoding trichodiene synthetase	2000	Mycologia	Volume 92, Number 4, pgs 764-771
000548 - 000556	Pedersen, P. B. ; Broadmeadow, A.	Toxicological studies on Thermomyces lanuginosus xylanase expressed by Fusarium venenatum, intended for use in food	2000	Food Additives and Contaminants	pgs 1-9

NA- Not applicable

AM




February 13, 2001

Rudaina Alrefai, Ph.D.
 Regulatory Policy Branch, HFS-206
 Division of Product Safety
 Center for Food Safety and Applied Nutrition
 Food and Drug Administration
 200 C Street SW
 Washington, DC 20204

Post-it® Fax Note 7671		Date 2/14/01	# of pages 1
To RUDAINA ALREFAI	From SCOTT SHORE		
Co./Dept. FDA/CFSA/OPA	Co. NOVOZYMES		
Phone #	Phone # 919 783-6348		
Fax # 202 418 3191	Fax #		

RE: GRAS Notification GRN#54 – Xylanase

Dear Dr. Alrefai,

This letter will serve to inform the Food and Drug Administration that Novo Nordisk A/S has de-merged into a health care company (Novo Nordisk A/S) and enzyme company (Novozymes A/S). The address for the worldwide corporate headquarters will continue to be Novo Allc, 2880 Bagsvaerd, Denmark.

As a result of this de-merger the name of the U.S. subsidiary has been changed from Novo Nordisk BioChem North America, Inc. to Novozymes North America, Inc. The correct address of the subsidiary is Novozymes North America, Inc., 77 Perry Chapel Church Road, P.O. Box 576, Franklinton, NC 27525.

In the event that you have questions or comments to the above, please do not hesitate to contact us.

Best regards,

Scott H. Shore, Ph.D.
 Sr. Regulatory Specialist

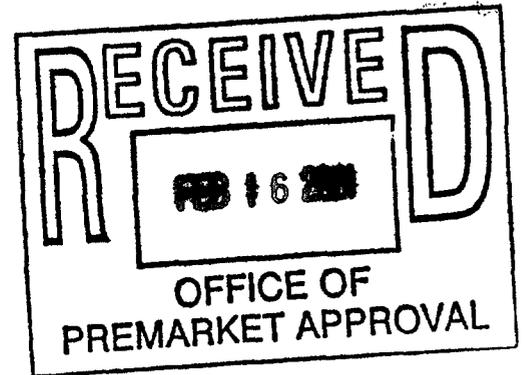
Novozymes North America, Inc.
 77 Perry Chapel Church Road
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000560



February 13, 2001

Rudaina Alrefai, Ph.D.
Regulatory Policy Branch, HFS-206
Division of Product Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
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Best regards,

Scott H. Shore, Ph.D.
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